A NEW MITE, *ICHORONYSSUS HUBBARDI,* FROM THE MOUNTAIN BEAVER, *APLODONTIA RUFA* (RAFINESQUE)

E. W. JAMESON, JR.

Division of Zoology, University of California, Davis

The following mite is the second species of the family LAELAPTIDAE described from the mountain beaver or sewellel; it is placed in the subfamily LIPONYSSINAE and in the genus *Ichoronyssus* Kolenati.

Ichoronyssus hubbardi, n. sp.

Female, Fig. 1

Body about twice as long as wide, and clothed with setae as illustrated. The legs are increasingly slender caudally, and bear several distinctive teeth which are usually setigerous.

Dorsum: Dorsal plate in one piece, incompletely covering dorsum, irregularly rounded caudally, with about twenty-five pairs of setae of relative size and position as illustrated; setae of dorsal plate generally longer laterally. Dorsal plate with small alate appendages adjacent to cephalic end of peritreme. The part of the dorsum not covered by the dorsal plate bears four or five pairs of setae, all caudal of the peritreme.

Peritreme short and arcuate, not extending in either direction beyond coxae III and IV.

Venter: Sternal plate poorly defined caudally, strongly arched. First and fourth pairs of sternal setae more slender than the second and third pairs. Genito-ventral plate rounded apically, separated from the anal plate by a distance approximately equal to the length of the latter. Genitoventral plate with one pair of setae which are slightly cephalad from the caudal borders of coxae IV. Anal plate of the shape as illustrated, twice as long as wide; the apex is frequently curved dorsad. The paired anal setae are cephalad from the middle of the anus, but this position is somewhat variable; in some specimens they are completely cephalad from the anus. The unpaired seta swollen proximally, about twice the length of the paired setae. Setae on unsclerotized part of venter tapered not gradually, but rather abruptly near tips.

Legs: Coxa I with a large, truncate, setigerous tooth ventrally. Coxa II with a smaller, rounded tooth, and a seta laterad from it. Coxa III with a bifid setigerous tooth. Coxa IV without teeth. Leg I with a large tooth (bearing three setae)

---

dorsally on the second segment and a setigerous tooth ventrally on the fourth segment.   Leg II with a large, rounded, setigerous tooth dorsally on the second segment and a small, pointed, setigerous tooth ventrally on the fifth segment.   Leg III with a small, blunt tooth ventrally on the fifth segment.   Leg IV without distinguishing teeth.
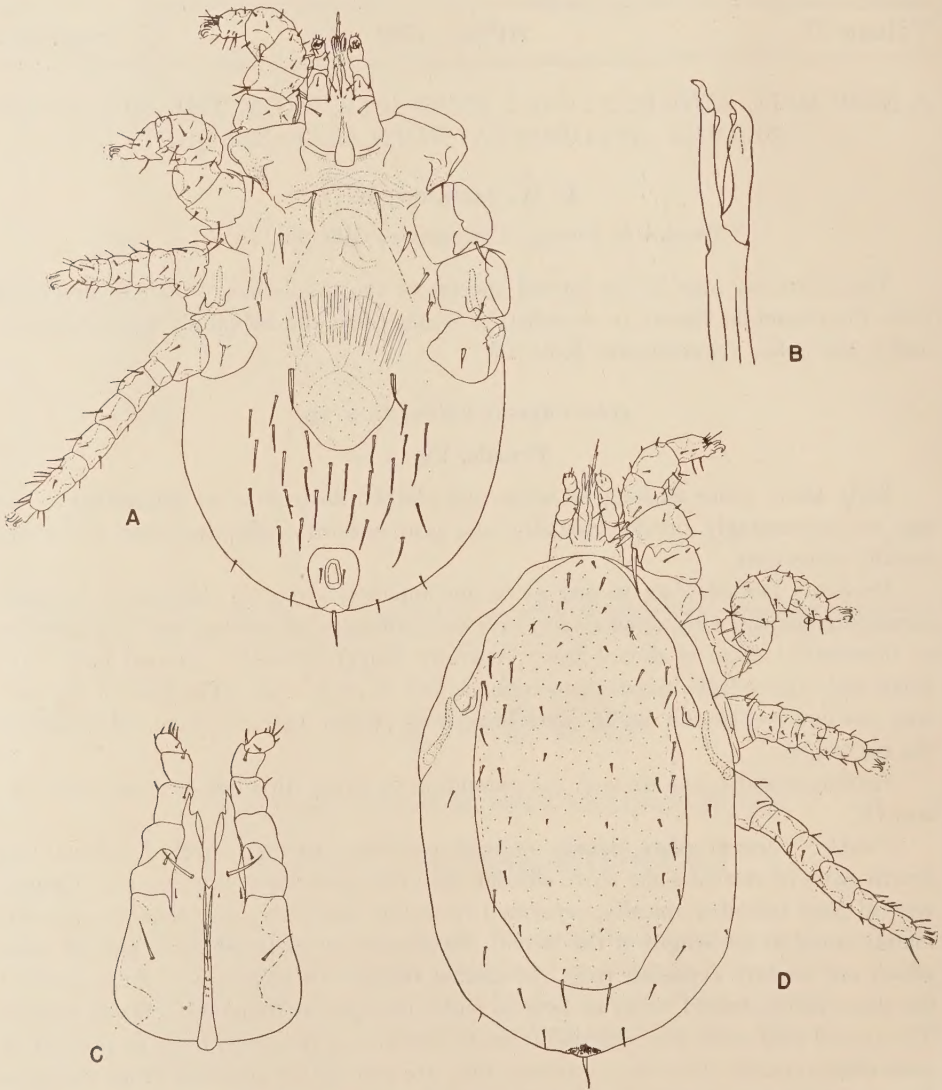

Fig. 1.   Female of *Ichoronyssus hubbardi,* n. sp. A, venter; B, chelicera; C, ventral view of gnathasoma; D. dorsum.

Gnathasoma: Palpi without any teeth or processes.   Ventral surface of gnathasoma with the usual four pairs of setae; of the two central pairs, the more mesal is the smaller.   Chelicerae shear-like.   Fixed chela longer than the movable one, with a seta at its base; movable chela with three low tubercles on its inner surface.

Male, Fig. 2

Smaller and more circular than the female.

Dorsum: Dorsal plate widest at middle, incompletely covering dorsum, with about twenty-six pairs of setae of which the lateral are the longer. Part not covered by dorsal plate with two pairs of setae.

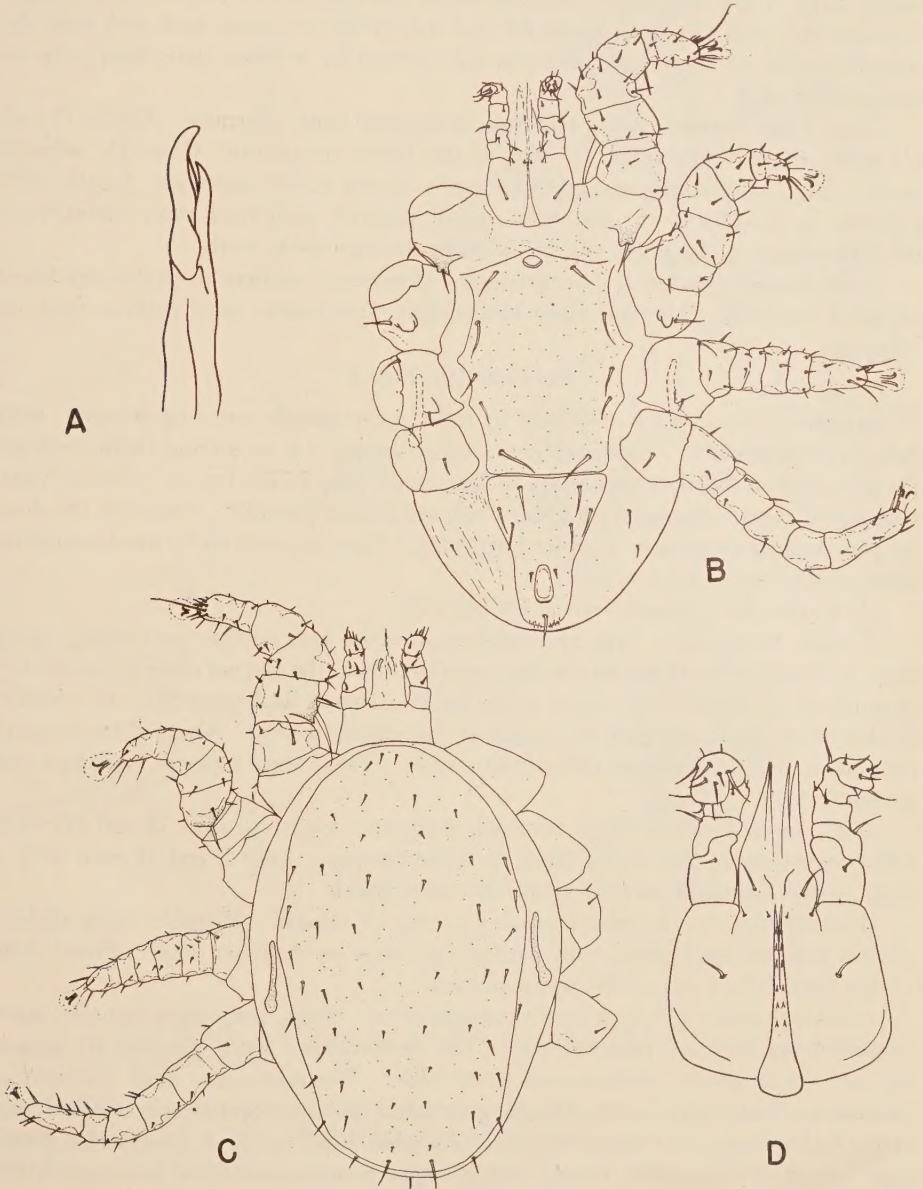Peritreme as in female, very short, above coxae III and IV.

FIG. 2. Male of *Ichoronyssus hubbardi,* n. sp. A, chelicera; B, venter; C, dorsum; D, ventral view of gnathasoma.

Venter: Ventral plate of two parts, separated by a transverse suture between coxae IV. Intercoxal plate bearing five pairs of setae on its periphery, widest between coxae II and III at which point it is produced laterally. The first pair of setae on the cephalic border of the intercoxal plate; second pair between coxae II; third and fourth pairs between coxae III; and the fifth pair (the longest) between coxae IV. The enlarged anal plate with a slightly concave cephalic border. Near each lateral angle of the anal plate are three setae arranged in a triangle. Paired anal setae laterad from the anus, one-third the size of the unpaired seta, and near the cephalic border of the anus. The area not covered by the two plates bears one or two pairs of setae.

Legs: Coxa I with a large, truncate, setigerous tooth ventrally. Coxae II and III each with a tooth ventrally, that of the latter setigerous. Coxa IV without tooth. Leg I with a setigerous tooth dorsally on the second segment. Leg II with a similar but smaller tooth; and with a small, pointed, setigerous tooth ventrally on the fifth segment. Legs III and IV without distinguishing teeth.

Gnathasoma: Similar to that of female. Proximal segment of palpus produced cephalad ventrally. Movable chela longer than fixed chela; both without teeth or tubercles.

Deutonymph, Fig. 3

Dorsum: Dorsal plate narrowed in front, incompletely covering dorsum, with deep fissures caudally. Cephalad from the peritreme is a projecting tooth, perhaps the precursor of the alate appendage on the dorsal plate of the female. Dorsal plate with about twenty-one pairs of setae which are longer laterally. Caudally the dorsal plate bears a single pair of rather long setae. Part not covered by the dorsal plate bears nine or ten pairs of setae.

Peritreme short, between coxae III and IV.

Venter: Sternal plate oval, twice as long as wide, with four pairs of setae. Anal plate widened and rounded anteriorly, separated from the sternal plate by a distance approximately equal to the length of the latter. Paired anal setae near the cephalic border of the anus, one-half the length of the unpaired seta. About thirteen pairs of setae on the unsclerotized part of the venter. These setae taper at their tips, not gradually from the bases.

Legs: Coxa I with a large, truncate, setigerous tooth. Coxae II and III each with smaller teeth, that of the latter being setigerous. Legs I and II each with a large, setigerous tooth dorsally on the second segment.

Gnathasoma: As illustrated, similar to that of female. Movable chela slightly shorter than the fixed chela, with a subapical fringe on the inner edge. Fixed chela with a basal fringe and a bifid apical process.

Collection data: The type series consists of two males, forty-eight females, eight deutonymphs, and two protonymphs. The protonymphs were distorted in mounting and no diagnostic features are discernible. The specimens were collected at Skamania, Skamania County, Washington from the mountain beaver (*Aplodontia rufa* (Rafinesque), APLODONTIIDAE), 28 October 1933 by C. A. Hubbard. Holotype female, allotype male, twenty-five paratype females, and three paratype deutonymphs are deposited at the United States National Museum. The remainder of the specimens are designated as paratypes, and deposited in the collection of the

author and with the following institutions or persons: British Museum (Natural History), London, England; Department of Entomology, Cornell University, Ithaca, New York; Rocky Mountain Laboratory, Hamilton, Montana; Instituto Butantan, São Paulo, Brazil; Natal Museum, Pietermaritzburg, South Africa; Dominion En-
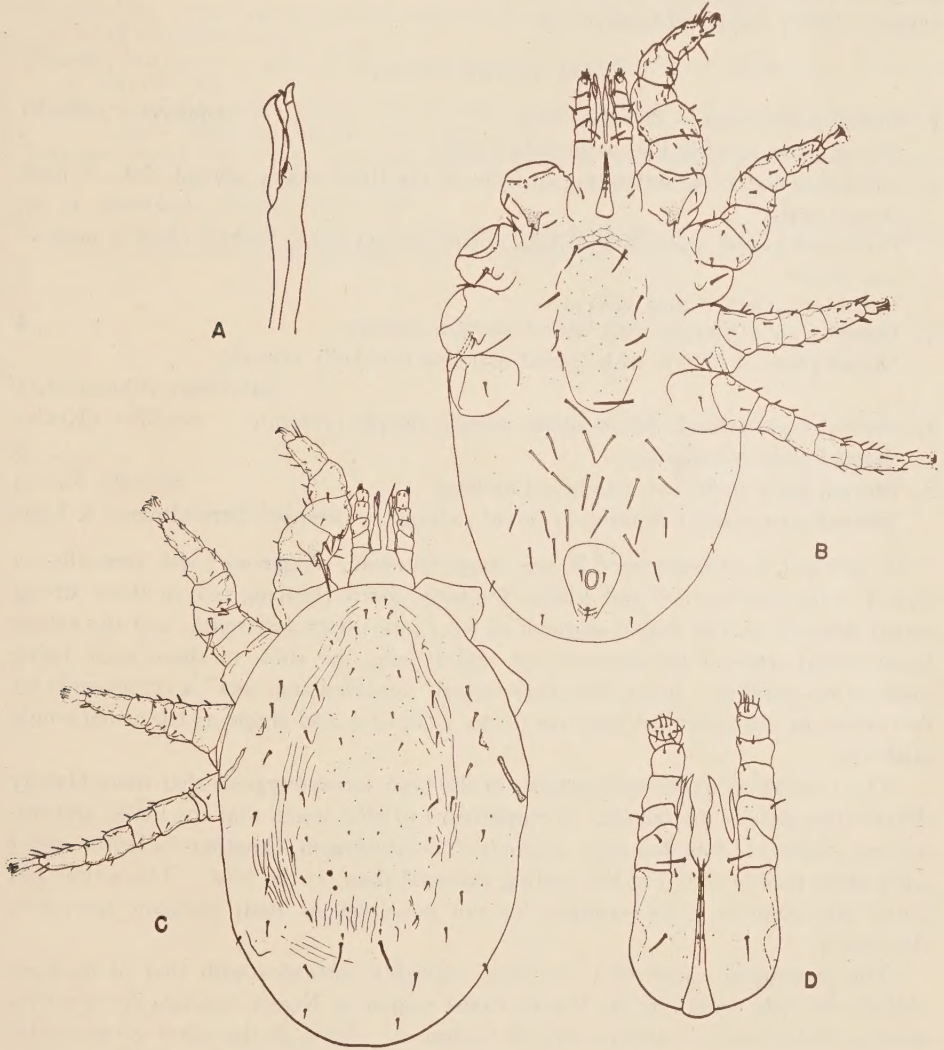
Fig. 3. Deutonymph of *Ichoronyssus hubbardi*, n. sp. A, chelicera; B, venter; C, dorsum; D, ventral view of gnathasoma.

tomological Laboratory, Kamloops, British Columbia, Canada; Laboratoire de Zoologie du Muséum, Muséum National d'Histoire Natural, Paris, France; Museum of Comparative Zoology, Cambridge, Massachusetts; Dr. Megumi Hasegawa, Research Institute, International Christian University, Tokyo, Japan; and Dr. R. W. Strandtmann, Texas Technological College, Lubbock, Texas.

Comments.—This mite does not closely resemble any other known species of *Ichoronyssus*. The North American species of this genus are being studied by Dr. R. W. Strandtmann, and a discussion of the relationships of *I. hubbardi,* n. sp. at this time would be premature. Ewing's key (1922: 13) to the North American species of *Ichoronyssus* can be modified as follows to include *I. dentipes* Strandtmann & Eads (1947) and *I. hubbardi,* n. sp.

KEY TO THE SPECIES

1. Dorsal plate about as broad as long .... *semitectus* (Koch)
   Dorsal plate scarcely half as broad as long 2
2. Peritreme short, not extending in front of the third coxa; ventral plate of male
   of two parts .... *hubbardi,* n. sp.
   Peritreme longer, extending at least to the second coxa; ventral plate of male of
   one piece* 3
   * Male of *sternalis* Ewing unknown.
3. Dorsal plate of female with lateral margins concave 4
   Dorsal plate of female with lateral margins decidedly convex.

   　　　　　　　　　　　　　　　　　isabellinus (Oudemans)
4. Sternal plate arched, the posterior margin deeply crecentic .. *carnifex* (Koch)
   Sternal plate rectangular 5
5. Sternal plate about twice as broad as long .... *sternalis* Ewing
   Sternal plate about five times as broad as long ... *dentipes* Strandtmann & Eads

I. hubbardi is characterized by the large, truncate, setigerous tooth ventrally on coxa I of the deutonymph and adults; the large tooth (bearing two or three strong setae) dorsally on the second segment of leg I; the short peritreme; and the rather long ventral setae of the deutonymph and female, the sides of these setae being more or less parallel. In one female the usual "unpaired anal seta" is represented by two setae; in this specimen they are of the same size and shape as the usual single anal seta.

The scarcity of males and nymphs in the type series suggests that their biology differs from that of the female. Strandtmann (1946) noted that the males and immature stages of *Atricholaelaps sigmodoni* Strandtmann (another laelaptid mite) were more readily found in the nesting material than on the host. The writer has found this situation to be common, but not universal, in many parasitic species of this family.

The geographic range of *I. hubbardi* probably coincides with that of its host. *Aplodontia rufa* occurs in the Pacific Coast region of North America from northwestern California to southern British Columbia. Some of the other ectoparasites of the mountain beaver are also very distinctive (Jellison, 1945). These include a beetle, *Leptinillus aplodontiae* Ferris; two fleas, *Dolichopsyllus stylosus* (Baker) and *Trichopsylloides oregonensis* Ewing; and two mites, *Laelaps aplodontiae* Jellison and *Trombicula aplodontiae* Brennan. The two genera of fleas are monotypic, and the species of mites are atypical representatives of their respective genera. The host is the only living species of ApLODONTOIDEA, the oldest group of rodents, whose history dates from the Upper Paleocene (Simpson, 1945).

This aberrant species bears the name of Mr. C. A. Hubbard who has done much

to stimulate the study of ectoparasites in this country. For comparing this species to specimens of *Ichoronyssus* in the U. S. National Museum I am grateful to Dr. E. W. Baker; and for helful criticism of the drawings I wish to thank Dr. R. W. Strandtmann.

REFERENCES

Ewing, H. E.   1922   The Dermanyssid mites of North America.   Proc. U. S. National Museum **62** No. 2459: 1–26.

Jellison, William L.   1945   A new mite, *Laelaps aplondontiae,* from *Aplodontia.*   J. Parasit. **31:** 373–374.

Simpson, George Gaylord   1945   The principles of classification and a classification of mammals.   Bull. Am. Mus. Nat. Hist. **85:** 350 pp.

Strandtmann, R. W.   1946   *Atricholaelaps sigmodoni,* a new species of mite parasitic on the cotton rat, and notes on the genera *Atricholaelaps* and *Ischnolaelaps* (Acarina: Laelaptidae).   J. Parasit. **32:** 164–169.

———— and R. B. Eads   1947   A new species of mite, *Ichoronyssus dentipes* (Acarina: Liponyssinae), from the cotton rat.   J. Parasit. **33:** 51–56.

HUMAN GNATHOSTOMIASIS IN SIAM WITH REFERENCE TO THE METHOD OF PREVENTION*

Svasti Daengsvang

Since Richard Owen (1836) first discovered and described *Gnathostoma spinigerum* from the stomach of a tiger, there have been many cases of human gnathostomiasis reported in the literature from many countries in the Far East, including the Malay States, China, Japan, India, and Siam. In all of these cases the worms were identified as *G. spinigerum,* except in the Japanese case in which the worm was considered to be a different species, *G. hispidum* (Morishita, 1924). A careful search of the available literature reveals that the first case of human gnathostomiasis was a Siamese woman in Bangkok reported by Levinsen (1889) who obtained the clinical history and the worm of the case from Deuntzer. The second case was also a Siamese woman reported by Leiper (1909) who received the worm from Dr. Kerr. Since then 15 more cases have been added from Siam of which one was a man and the rest were women (Robert, 1922; Prommas and Daengsvang, 1934; Rhithibaed and Daengsvang, 1937; Daengsvang, 1939; Sirisamban, 1941).

During the years 1942 to 1947, 17 more Siamese patients suffering from gnathostomiasis, 2 males and 15 females, were seen at the Siriraj Hospital, Bangkok. These findings suggest that this infection is not at all rare in Siam, and indicate the need of further studies on its prevalence and etiology.

DISCUSSION OF CASES

These 17 new cases of gnathostomiasis will be briefly described in five groups according to the symptoms.

Group 1. Six patients, one man and five women, of various ages (18 to 80) had common symptoms characterized by migrating intermittent swellings of different sizes on the skin of different parts of the body. These swellings were most frequently on the chest and abdomen, but occasionally on the hand and arm (Fig. 1). The swelling was sometimes itchy, painful and tender, but showed no pitting on pressure. The pain, if present, was a mild boring or pricking sensation of short duration. A few small hemorrhagic spots were sometimes seen with very small cord-like masses in the swelling. These patients had no fever and their blood pictures showed an eosinophilia of varying degrees (5 to 73%). Two immature gnathostomes escaped spontaneously from the swellings of the skin of each of two cases and one immature gnathostome was removed with a piece of skin by surgical excision from each of the other 4 cases. The immature worms after fixation with 70% alcohol ranged in size from 2.7 to 6.5 mm. by 0.4 to 1.5 mm., and showed 4 to 7 rows of cephalic spines. Although each attack of the disease did not last more than ten days, one case had had more than 6 attacks in one year and had suffered for a period of 3 years before the worm had escaped spontaneously from the skin of the head.

Group 2. There were 3 cases (1 man and 2 women) in this group with histories

of migrating intermittent swellings of the skin of the abdomen and face accompanied by mild boring pains and intermittent itching lasting for periods of a few days to 3 months. One of them suddenly experienced a moderate degree of headache with considerable swelling of the eyelids and a moderate degree of ecchymosis of the right eye before an immature gnathostome of about 6 mm. in length could be removed from the iris. This case showed permanent impairment of the vision of the right eye on account of scar formation in the iris muscle (Fig. 2). The two other cases suffered from intermittent swelling of lips, cheeks and finally of the eyelids for a few weeks which later was followed by a moderate degree of boring pain and disturbances in the vision of the right eye. A small worm could be easily seen with the naked eye in the eye-ball of each case, and an immature gnathostome was successfully removed from the anterior chamber of one case and from the iris muscle of the other (Fig. 3). The symptoms in these two cases began to subside rapidly and the vision became normal in a week. The blood pictures were normal except for an eosinophilia of 3 to 46%.

Group 3. This group comprised 3 female cases who had more or less severe coughs accompanied by a slight degree of difficulty in breathing. In one of them bilateral subconjunctival hemorrhage was present after frequent severe attacks of a dry cough for almost the whole night; at last an immature gnathostome was coughed up in the morning of the next day and the condition began at once to subside. The next case had a previous history of acute right pleurisy lasting for about one week; this was later followed by difficulty in breathing, and finally an immature gnathostome was coughed up spontaneously. The last case had for a few weeks a frequent intermittent migrating swelling of the right arm and shoulder accompanied by slight pain and itching; then there were symptoms of meningeal irritation, especially headache, intermittent convulsions, and semi-unconsciousness lasting for about 7 days; finally a fully developed male *Gnathostoma spinigerum* measuring 12.0 mm. in length with 8 rows of cephalic spines was coughed up, after which there was complete recovery. Blood pictures of these 3 cases showed eosinophilia of 30%, 53% and 8% respectively.

Group 4. This group consisted of 4 female patients aged 25 to 44. Three of them had previous clinical histories lasting for 2 to 3 years of migrating intermittent swellings of the skin of many parts of the body and lastly of the anterior abdominal wall. The swelling was always accompanied by a mild degree of pain and itching. Finally each of these cases had felt a small movable intra-abdominal tumor in the right lower part of the abdomen for a period of about 3 months before coming for the operation. The tumor was slow growing and tender. The general health of each patient was normal. Blood pictures of the 3 cases were normal except for 16%, 28%, and 48% eosinophilia. Surprisingly enough after laparotomy, each patient revealed a smooth, hard, more or less rounded tumor of the omentum adhering to the ileocecal part of the intestine. They were all about the same size (5 cm. in diameter, Fig. 4). One gnathostome was found in a small necrotic space in each tumor. There were two fully developed male worms and one immature female, each with 8 rows of cephalic spines; one of the male worms measured 15.0 mm. by 0.95 mm. and the female measured 10.2 mm. by 0.65 mm. after fixation with warm 70% alcohol.
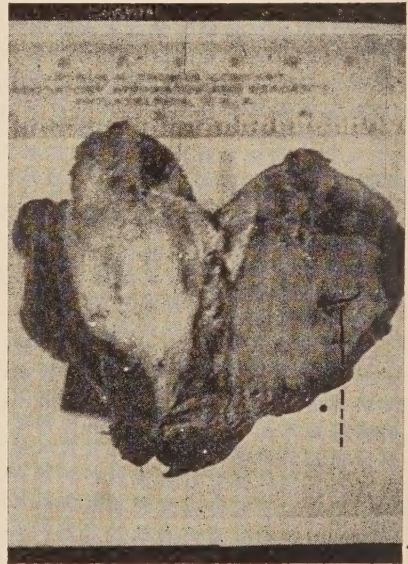
DESCRIPTIONS OF FIGURES

FIG. 1.   Swelling of the right hand and fore-arm caused by *Gnathostoma spinigerum*.

FIG. 2.   A case of ocular gnathostomiasis in a man.   An immature gnathostome was successfully removed from the iris muscle.

FIG. 3.   Another case of ocular gnathostomiasis in a woman with considerable swelling of eyelids and surrounding tissues.

FIG. 4.   A tumor removed from the omentum adhering to the ileo-cecal part of the intestine.   A fully developed *Gnathostoma spinigerum* can be seen in a necrotic space inside the tumor.

The fourth case of group 4 had no clinical history of migrating intermittent swelling but developed an intra-abdominal tumor about the size of a hen's egg which was slightly movable and tender in the gastro-colic region. Her blood picture was normal except for 22% eosinophilia. The tumor after surgical removal was more or less smooth and rounded with hemorrhagic areas here and there on its periphery. A fully developed male of *Gnathostoma spinigerum* with 8 rows of cephalic spines was obtained from a small necrotic space inside the tumor.

Group 5. The fifth group comprised only one female case who also had suffered for a period of a few weeks from migrating intermittent swellings of the face accompanied by a mild degree of boring or pricking pain and intermittent itching. Finally an immature gnathostome with 4 rows of cephalic spines was removed by a friend with a needle point from a small swelling of the soft palate. About 10 days later another immature gnathostome of about the same size escaped spontaneously from a small painful swelling on the upper part of her right thigh. Her blood picture showed 52% eosinophiles.

Discussion. In addition to the symptoms described above hematuria and leukorrhea of moderate degree have been observed in some patients by other doctors when the worms migrated into the bladder or uterus respectively; these symptoms disappeared at once when the worms were removed.

The tissue lesions caused by gnathostome infection usually show many areas of degeneration and necrosis together with heavy infiltration by a great number of eosinophilic and neutrophilic polymorphonuclear leucocytes; at some places hemorrhagic exudates and mononuclear cells are also present. The tissue which is continuously affected by the worm usually shows fibrous formation together with much cellular infiltration.

The diagnosis of gnathostomiasis in Siam is not very difficult because up to the present time there are no other diseases having the clinical symptoms of migrating intermittent swelling of the skin and mucous membranes sometimes with boring or pricking pain and itching. Frequently such symptoms occur and gnathostomiasis is suspected without confirmation by recovery of the worms. The blood picture in most cases shows a high percentage of eosinophiles. Usually the general body health of the patient is not much affected and the prognosis is very good unless the worm migrates into and damages some vital organ.

The most reliable method of treatment for permanent cure of the disease is the surgical removal of the worm together with the diseased tissue. Antimony compounds in the forms of fouadin and anthiomaline were tried in some cases but with uncertain results.

Finally, I am of the opinion that human gnathostomiasis is rather common in Siam. Unreported cases have been observed in hospitals and by local physicians, and suspected cases in which worms have not been found are not infrequently seen.

ETIOLOGY AND PREVENTION

In order to have a clear idea on the preventive measures that might be used against human gnathostomiasis in Siam the life-cycle, which was worked out by Prommas and Daengsvang (1933, 1936 and 1937), will be reviewed briefly.

The adults of *Gnathostoma spinigerum* are normally found in the stomach wall

of the cat and dog in Siam.   The female lays undeveloped eggs into the lumen of the digestive tract which pass out with the animal's excreta.   The eggs under optimum temperature and moisture become embryonated and then the embryos escape through the knob of the egg shell as free-living larvae (Prommas and Daengsvang, 1933, Figs. 1–3).   This first stage larva is then taken up by a cyclops in which it undergoes further development up to the size of about 372 by 62 microns; the cuticle at this stage is transversely striated and its anterior one-fourth is covered with transverse rows of minute single-pointed spines.   The cephalic bulb is definitely developed and is covered with 4-transverse rows of many single-pointed spines (1.c. Figs. 4a and b).   In order to become infective to the cat, the second stage larvae must be ingested by fresh-water fish.   In the flesh and visceral organs of the fish they increase greatly in size and become surrounded by a cyst, about 1.14 mm. in diameter. Here they may reach a size of about 2.7 by 0.29 mm. Table 1 lists the second intermediate hosts that have been found infected in Siam (Daengsvang and Tansurat, 1938; Tansurat, 1947).   Africa and co-workers (1936) have also found the en-

TABLE 1.—*Shows fresh-water animals found by Daengsvang and Tansurat as natural second intermediate hosts of* Gnathostoma spinigerum *in Siam*

Species of fresh-water fish and other animals	% infected
Clarias batrachus (Linnaeus)	30
Ophicephalus striatus (Bloch)	37.5
Ophicephalus micropeltes (Curv. & Val.)	19
Notopterus chitala (Buchanan)	11
Anabas testudineus (Bloch)	5
Trichopodus trichopterus (Pallas)	3
Trichopodus pectoralis (Regan)	2
Crypteropus apongon (Bleeker)	2
Monopterus albus (Zuieuw)	80
Rana rugulosa (Wiegmann)	91.7
Fresh-water snake (species unidentified)	100

cysted gnathostome larvae in the Philippine Islands in 25% of *Ophicephalus striatus,* 100% of *Glossogobius giuris,* and 12.6% of *Therapon argenteus.*

The development of the adult worm in the wall of the cat's stomach usually takes place within six and one-half months after the infective larva has been ingested with fish, and it takes about 8 months to complete the life-cycle under suitable conditions.   There is little doubt that human infection is acquired from fish.   Tansurat and I have recently found many living infective gnathostome larvae in a kind of fermented food bought from local markets which is mainly made of the raw flesh of fresh-water fish, *Ophicephalus striatus,* etc.   The gnathostome larvae found in this food after being fed to a cat developed to adult worms in the stomach wall.   This dish is a favorite of women which probably explains the higher incidence of the gnathostomiasis among Siamese women.

It was found that a living infective larva which was 1 cm. deep in the muscle of a fish could be killed by being treated for at least 5 minutes in boiling water.   The infective larvae could also be killed after being left in vinegar which contained 4% acetic acid for five and one-half hours, but they were found alive in lime juice after 5 days at room temperature.   The larvae were still alive after being kept at a temperature of 4° C. for one month.   It appears, therefore, that the way to prevent infection with *G. spingerum* is to avoid all dishes that contain raw or poorly cooked fresh-water fish.

SUMMARY

Seventeen cases of gnathostomiasis collected from the records of the Siriraj Hospital during the years 1942 to 1947 are added to the seventeen previously reported from Siam. They are discussed in five groups according to symptoms. The diagnosis of the disease in Siam can be made by the use of clinical histories and symptoms comprising chiefly migrating, intermittent swelling of the skin, and sometimes of mucous membranes, with a mild degree of boring or pricking pain and itching; the blood picture shows more or less eosinophilia. In addition, some other symptoms are present which result from injury of the affected organs. The tissue lesions caused by the parasite and the treatment of the disease are also briefly mentioned. The life-cycle of *Gnathostoma spinigerum* is briefly reviewed and all species of fresh-water animals which are known to serve as second intermediate hosts of the worm in nature in Siam are listed. Certain dishes containing the flesh of fresh-water fish that harbor this parasite are commonly eaten raw or partially cooked in Siam. It was demonstrated that larvae found in these foods produced infection in experimental animals. The only sure method of prevention is to cook thoroughly the flesh of any fresh-water fish that is to be used for food.

LITERATURE CITED

AFRICA, C. M., REFUERZO, P. G. AND GARCIA, E. Y. 1936 Observations on the life-cycle of *Gnathostoma spinigerum*. Philippine J. Sci. **59**: 513–521.

DAENGSVANG, S. 1939 An abdominal tumor caused by *Gnathostoma spinigerum* (Owen, 1836). Indian Med. Gaz. **74**: 399.

———— AND TANSURAT, P. 1938 A contribution to the knowledge of the Second Intermediate Hosts of *Gnathostoma spinigerum* (Owen, 1836). Ann. Trop. Med. Parasit. **32**: 137–140.

LEIPER, R. T. 1909 The structure and relationships of *Gnathostoma siamense* (Levinsen). Parasitol. **2**: 77–80.

LEVINSEN, G. M. R. 1889 Om en ny Rundom hos mennesket Cheiracanthus siamensis n. sp. Vidensk. meddel. fra naturk. Foren. i. kjbenhaven. f. 1889, p. 323.

MORISHITA, K. O. R. 1924 A pig nematode *Gnathostoma hispidum* Fedchenko as a human parasite. Ann. Trop. Med. Parasit. **18**: 23–26.

OWEN, R. 1836 Anatomical descriptions of two species of Entozoa from the stomach of a tiger (*Felis tigris* Linn.), one of which forms a new genus of Nematoidea, *Gnathostoma*. Proc. Zool. Soc. Lond. **47**: 123–126.

PROMMAS, C. AND DAENGSVANG, S. 1933 Preliminary report of a study on the life-cycle of *Gnathostoma spinigerum*. J. Parasit. **19**: 287–292.

———— AND ————1934 Nine cases of human gnathostomiasis. Indian Med. Gaz. **69**: 207–210.

———— AND ————1936 Further report of a study on the life-cycle of *Gnathostoma spinigerum*. J. Parasit. **22**: 180–186.

———— AND ————1937 Feeding experiments on cats with *Gnathostoma spinigerum* larvae obtained from the second intermediate host. J. Parasit. **23**: 115–116.

RHITHIBAED, C. AND DAENGSVANG, S. 1937 A case of blindness caused by *Gnathostoma spinigerum* (in Siamese). Med. J. Med. Ass. of Siam. **19**: 840–845.

ROBERT, P. L. 1922 La Gnathostomose Humaine. Oedème ambulant siamois dû à *Gnathostomum spinigerum* (R. Owen, 1836). Bull. Soc. Path. Exot. **15**: 854–860.

SIRISAMBAN, B. S. 1941 A case of ocular gnathostomiasis. (in Siamese) Med. J. Med. Ass. of Siam. **24**: 401–408.

TANSURAT, P. 1947 Foods which carry the ineffective gnathostome larvae to human beings. (in Siamese) Thesis submitted to the Faculty of Medicine and Siriraj Hospital, for the Degree of Doctor of Medicine in 1947.

DICYEMA SULLIVANI, A NEW MESOZOAN FROM LOWER CALIFORNIA*

Bayard H. McConnaughey

The Dicyemidae from American waters have long remained unknown, the only published references to them being two short preliminary papers by Wheeler (1897, 1899), two by McConnaughey (1938, 1941) and part of the recent monograph by Nouvel (1947). For the past two years the writer has been engaged in a study of the dicyemids at the Scripps Institution of Oceanography, La Jolla, California. The results are embodied in a Ph.D. thesis submitted to the University of California (McConnaughey, 1948). In this work twelve species of dicyemids, including four belonging to the genus *Dicyema,* from the California coast are described.

A paper containing keys, descriptions and figures for these species has been submitted for publication. Subsequent to the preparation of this article, an additional species has been found in a small octopus from Lower California.

Dicyema sullivani n.sp.

Diagnosis: Adult nematogens usually from three-fourth to one and one-half millimeters long, slender, of about the same width throughout or with a moderate cephalic swelling broadest at the level of the paraporal cells; total number of somatic cells 28 to 33, most commonly 32; trunk very uniform in appearance, without verruciform cells or heavy accumulations of granules, the cells disposed partly in opposed pairs, partly in whorls of four; cephalic swelling darker staining than the trunk; calotte orthotropal, conical, tapering somewhat to the bluntly rounded anterior end; propolar cells about two-fifths to one-half the size of the metapolar cells; parapolar cells shield shaped, about equal in length to the length of the calotte; axial cell ending bluntly between the bases of the metapolar cells, not extending forward to the propolars.

The vermiform larvae attain a length of about 100 microns before escaping from the parent nematogens. In them the calotte is elongated, pointed, and appears to be twisted spirally a quarter of a turn so that the cells often appear to be diagonal in position (figs. 6 and 7). The axial cell ends anteriorly near the posterior ends of the metapolar cells. The first axoblasts divide early in this species so that typically four or even more are present in the larva before its escape. The axoblasts tend to remain contiguous for some time both in the larvae and in young nematogens, often forming small clusters of cells.

The axial cell nucleus may give rise by amitotic division to one or two additional nuclei, usually smaller (fig. 1).

Host: *Octopus bimaculatus* Verrill.

Distribution: At present known only from the northern part of Lower California.

Type specimens: Syntypes on slide L-14,-2, author's collection. Paratypes on some of the other slides of the L-14 series.

This species was found in one of five immature octopuses weighing from three-

fourths to two and one-half grams, collected on a rocky flat near Rosarita Beach, Mexico, in the intertidal zone. All five of them contained nematogens of *Dicyemennea california* McConnaughey, 1941, one of them being infected in addition with the species here described. The two species were located in separate clusters on the kidney lobules, not being randomly intermixed. Only the primary nematogen phase of both species was present.

This species differs from the majority of its congeners in the fact that the axial cell terminates at the posterior end of the calotte rather than penetrating forward between the metapolar cells to the propolar cells. The other species of *Dicyema* known to exhibit this condition (*D. acheroni* and *D. acciaccatum* McConnaughey, in press, and *D. monodi* Nouvel, 1934, and *D. acuticephalum* Nouvel, 1947) are all smaller with a lower somatic cell count, and they also differ in the size and shape of the calotte and trunk.

SUMMARY

Dicyema sullivani from Lower California, a parasite of *Octopus bimaculatus* is described. Its most distinctive features are the ending of the axial cell at the posterior border of the calotte, the large somatic cell count and the early multiplication of axoblasts in the vermiform larvae.

This brings to 13 the number of species of dicyemids known to occur along the west coast of North America, 5 of them belonging to the genus *Dicyema*, 7 to *Dicyemennea* and 1 to *Conocyema*.

REFERENCES

McConnaughey, B. H. 1938 The Dicyemid Mesozoa. Pomona College Journal of Entomology and Zoology. March: 1–14.
————1941 Two new Mesozoa from California, *Dicyemennea californica* and *Dicyemennea brevicephala* (Dicyemidae). J. Parasit. **27**: 63–69.
————1948 A study of the dicyemid Mesozoa. A thesis submitted to the University of California in partial satisfaction of the requirements for the degree of Doctor of Philosophy.
Nouvel, H. 1947 Les Dicyémides. Arch. Biol. **58**: 59–220.
Wheeler, W. M. 1897 Marine fauna of San Diego Bay, California. Science n.s. **5**: 775–776.
————1899 The Life History of *Dicyema*. Zool. Anz. **22**: 169–176. (Summary in Science n.s. **9**: 369–370).

EXPLANATION OF THE PLATE

All figures made with the aid of a camera lucida from preparations fixed in Bouin's fluid and stained with Ehrlich's acid hematoxylin.

Fig. 1 Nematogen containing vermiform larvae in various stages of development. The axial cell nucleus and two small accessory nuclei present in the axial cell are hatched to distinguish them from the somatic cell nuclei.

Fig. 2. Anterior end of a nematogen in side view, showing the calotte and parapolar cells.

Fig. 3. Anterior end of a nematogen in dorsal view.

Figs. 4 and 5. Very young emerged nematogens prior to the formation of vermiform larvae.

Figs. 6 and 7. Vermiform larvae still inclosed in the axial cells of their parent nematogens. Note the spiral twist of the calotte, and the four axoblasts already present in the axial cell. One of the larvae has 31 somatic cells, the other 33.

QUANTITATIVE HOOKWORM DIAGNOSIS
BY DIRECT SMEAR[1]

PAUL C. BEAVER

INTRODUCTION

That egg counts per direct fecal smear can be used for rough estimation of hookworm burden was demonstrated by Smillie (1921), Cort and Payne (1922), and Keller (1934). However, in the absence of definitive evidence that hookworm eggs are uniformly distributed in the fecal mass, confidence was lacking in counts based on such minute samples as are contained in direct smears. Moreover, ordinary fecal smears are not uniformly made and, therefore, do not represent uniform samples of feces. That hookworm eggs are indeed more or less uniformly distributed in the fecal mass was suggested by Hausheer and Herrick (1926) who found that the direct fecal smear in the hands of experienced technicians is diagnostically accurate if the stool contains a minimum of 300–500 eggs per gram. To determine definitely the threshold of accuracy at this low level for ordinary unstandardized fecal smears would necessarily require a high degree of uniformity in the distribution of the eggs. When in connection with another problem it was found that standardization of the direct smear could be based upon photoelectric measurement of density (turbidity) of the smear suspension, the problem of determining the reliability of direct smear egg counts as compared with counts made by an accepted standard method presented itself.

MATERIALS AND METHODS

Direct smears were made in much the usual manner but were made to a standard density as measured by a photoelectric foot-candle meter. Two different models and four different instruments were used, all of which have 40 mm circular windows to the photoelectric cell. This 40 mm aperture was reduced to a convenient size by fitting it with a wooden block 18 mm in thickness and drilling a central hole 16 mm ($\frac{5}{8}$ inch) in diameter. The inner surface of this tubular aperture was blackened with India ink. An electric lamp (75–100 watts) was suspended directly over the light meter and made vertically adjustable so that reading of about 20 foot-candles could be obtained with the light source placed 15–25 cm above the meter (figure 1).

One drop of water (0.045–0.050 cc) was placed near the center of a clean slide. With a wooden applicator a fleck of feces was taken at random from the stool sample and stirred into the drop of water until an even suspension of nearly proper density was produced. It was then placed on the meter and the light adjusted to give 20 foot-candles through a clean portion of the slide. Next, the smear was adjusted to the center of the window, spread carefully just to the periphery, and feces added until the light was reduced to exactly 10 foot-candles. Seeds, sand, course fiber, and other forms of gross debris were always removed from the smear before it was

made up to the standard density. When the smear was of an even suspension and of proper density, it was covered with a 22 x 22 mm coverglass and the eggs counted in the whole preparation.

Experience to date has shown that for satisfactory preparations it is necessary (1) to have the light source directly over the window of the meter; (2) to use only clean slides; (3) to take only just enough fecal material on the applicator to make the smear; (4) to use only pure feces in making the smear and break up all particles into an even suspension; (5) to adjust the light to exactly 20 foot-candles when read through the clear slide; (6) to spread the suspension to fit the 16 mm window, then
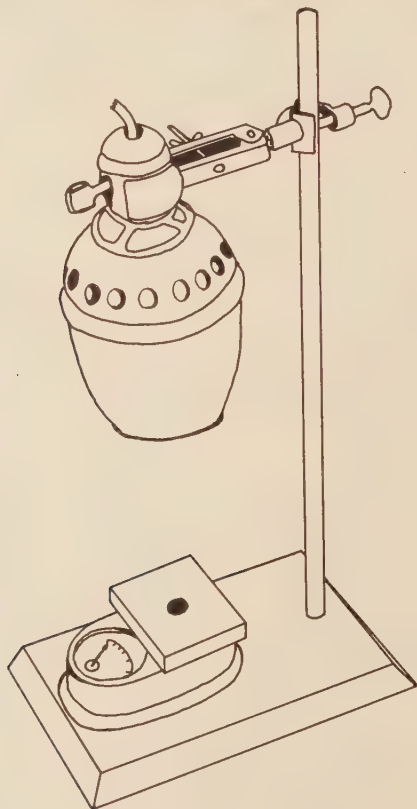
Fɪɢ. 1. Apparatus used for making fecal smears of uniform density.

add feces to reduce the light to exactly 10 foot-candles; and (7) to accept only preparations that can be counted easily and with confidence. If the slide should dry and air bubbles creep under the coverglass to confuse the counting, the preparation should be discarded.

The several different light meters used in this study were all adjusted to give readings equal to the first instrument used. For this calibration, fecal smears were used and comparisons made directly. Thus far it has not been possible to develop a satisfactory standard for independent exact calibration. However, for rough calibration of an assembly such as was developed for this study, a single thickness of ordinary microscope lens paper mounted dry under a No. 2 coverglass can be used as an

approximate equivalent to the standard direct smear in its light-screening qualities. Uncovered fecal smears would be made to reduce meter readings as much as the mounted lens paper. Also, one drop of Higgins India ink diluted either one drop of ink in 75 drops of distilled water, or one cc in 150 cc of distilled water, will give light reduction readings approximately equal to the standard direct smear suspension, when each is, of course, spread evenly over the 16 mm window of the mask. For the dilution of ink by the drop method, the size of the dropper is not important, but it is requisite that the same dropper be used for both the ink and the water.

Hookworm positive stools were obtained from two principal sources: (1) specimens received by mail for routine diagnosis, and (2) stools collected fresh from an orphange. As specimens were received, negatives were screened out by brine flotation and the positives set up in Stoll dilution flasks for egg counting on the following day. Direct smear counts were generally made the same day the specimens were

TABLE 1.—*Counts on stools from ten different individuals by the direct smear and dilution methods*

Stool no.	Successive counts of eggs per direct smear preparation	Average eggs per slide	Coefficient of variation	Successive counts of eggs per slide on small drop (0.075 cc.) dilution preparations	Average eggs per slide	Coefficient of variation
1.	9, 6, 7, 7, 6, 9, 8, 5, 10, 10	7.7	22	9, 3, 6, 7, 9, 4, 8, 5, 8, 5	6.4	31
2.	66, 64, 74, 78, 87, 75, 54, 61, 59, 75	69.3	14	44, 41, 67, 59, 50, 49, 62, 44, 62, 54	53.2	16
3.	17, 14, 18, 12, 16, 12, 13, 19, 15, 19	15.5	17	18, 12, 13, 9, 9, 14, 10, 10, 10, 8	11.3	25
4.	18, 18, 18, 21, 21, 30, 20, 31, 21, 21	21.9	20	19, 22, 12, 16, 14, 24, 20, 22, 19, 14	18.2	21
5.	62, 51, 34, 45, 58, 36, 46, 44, 35, 48	45.9	19	25, 24, 25, 23, 19, 24, 31, 33, 23, 33	26.0	17
6.	11, 12, 12, 10, 11, 9, 16, 10, 13, 12	11.6	16	16, 16, 14, 25, 19, 24, 17, 13, 14, 16	17.4	22
7.	19, 14, 12, 11, 15, 15, 16, 17, 10, 11	14.0	20	15, 18, 14, 10, 11, 15, 14, 12, 17, 10	13.6	20
8.	36, 27, 23, 28, 22, 18, 35, 28, 28, 24	26.9	20	24, 24, 25, 36, 17, 20, 24, 24, 25, 23	24.2	17
9.	19, 26, 9, 15, 19, 14, 22, 18, 21, 26	18.9	27	30, 24, 25, 33, 25, 17, 22, 19, 27, 35	25.7	21
10.	12, 22, 14, 11, 10, 12, 16, 14, 9, 17	13.7	27	10, 6, 10, 9, 9, 8, 12, 10, 6, 11	9.1	21
	Average	24.5	20		20.5	21

received. Any that were held over were kept under refrigeration to maintain fresh condition. In making the dilution counts, all those precautions described by the authors who originated and evaluated the dilution method were heeded (Stoll, 1923a; Stoll and Hausheer, 1926a, b). The dilution suspensions were set up and the stool consistency classifications made by an assistant who had been carrying out this routine procedure for the previous five years. All of the counts reported in tables I and II were made by the author. Some of the counts on cases II and III (table III) were made by a relatively inexperienced technician.

<center>OBSERVATIONS</center>

Variability of direct smear egg counts. Five or ten successive direct smear counts and an equal number of dilution counts were made on a series of different types of stools representing different intensities of infection. The two types of counts were then compared with reference to variabilty, and since the reliability of the dilution method is well established, (Stoll and Hausheer, 1926a; Soper, 1926), a measure of the reliability of the direct smear counts was thus obtained.

TABLE 2.—*Specimens on which 5 parallel counts were made by direct smear and dilution methods*

Stool No.	Successive counts by direct smear	Average	Coefficient of variation	Successive counts by dilution method	Average	Coefficient of variation
1.	21, 16, 25, 19, 19	20.0	15	20, 13, 11, 20, 12	15.2	26
2.	19, 25, 16, 22, 20	20.4	15	11, 13, 6, 8, 9	9.4	26
3.	55, 48, 78, 47, 57	57.0	20	39, 52, 58, 55, 57	52.2	13
4.	9, 11, 11, 16, 21	13.6	32	12, 14, 12, 18, 18	14.8	18
5.	14, 11, 10, 14, 13	12.4	13	7, 10, 8, 14, 13	10.4	26
6.	4, 9, 4, 5, 8	6.0	35	3, 6, 9, 5, 4	5.4	38
7.	15, 10, 13, 15, 11	12.8	16	39, 24, 29, 32, 22	29.2	21
8.	19, 15, 27, 18, 20	19.8	20	5, 6, 13, 11, 3	7.6	50
9.	27, 34, 24, 23, 29	27.4	14	9, 11, 10, 4, 7	8.2	30
10.	12, 10, 17, 22, 16	15.4	27	19, 30, 24, 26, 22	24.2	15
11.	8, 16, 24, 19, 20	17.4	31	9, 9, 5, 9, 4	7.2	31
12.	76, 60, 56, 90, 58	68.0	19	48, 63, 52, 47, 49	51.8	11
13.	69, 52, 49, 74, 60	60.8	16	65, 62, 60, 68, 64	63.8	4
14.	27, 38, 39, 34, 43	36.2	15	13, 21, 10, 5, 10	11.8	45
15.	33, 41, 37, 37, 43	38.2	9	44, 44, 38, 44, 43	42.6	6
16.	9, 8, 14, 15, 16	12.4	26	6, 10, 4, 11, 12	8.6	36
17.	2, 3, 8, 4, 2	3.8	59	2, 6, 5, 4, 6	4.6	32
18.	39, 39, 41, 48, 34	40.2	11	36, 42, 44, 48, 42	42.4	9
19.	68, 66, 84, 57, 56	66.2	15	39, 48, 42, 36, 30	39.0	15
20.	57, 49, 40, 43, 54	48.6	13	31, 25, 32, 26, 26	28.0	10
21.	9, 8, 10, 8, 12	9.4	16	2, 12, 10, 11, 3	7.6	56
22.	15, 13, 12, 9, 8	11.4	23	14, 10, 12, 10, 13	11.8	14
23.	20, 20, 26, 13, 25	20.8	22	14, 14, 8, 11, 10	11.4	20
24.	4, 7, 7, 7, 9	6.8	24	10, 15, 6, 7, 12	10.0	33
25.	4, 8, 7, 3, 4	5.2	37	2, 4, 4, 4, 5	3.8	26
26.	24, 32, 29, 22, 25	26.4	14	21, 22, 26, 25, 27	24.2	10
27.	14, 11, 9, 13, 16	12.6	19	4, 8, 9, 10, 6	7.4	29
28.	13, 6, 11, 8, 6	8.8	32	13, 7, 7, 10, 5	8.4	33
29.	16, 17, 19, 13, 19	16.8	13	36, 27, 40, 39, 39	36.2	13
30.	46, 57, 59, 62, 51	55.0	10	64, 61, 63, 57, 52	59.4	7
31.	10, 10, 9, 6, 14	9.8	26	2, 1, 6, 6, 1	3.2	72
32.	27, 18, 25, 22, 26	23.6	14	40, 46, 43, 37, 37	40.6	9
33.	6, 5, 3, 5, 4	4.6	22	3, 7, 6, 8, 6	6.0	28
34.	4, 4, 3, 4, 1	3.2	36	6, 3, 4, 4, 5	4.4	23
35.	7, 3, 3, 6, 6	5.0	34	13, 10, 9, 6, 7	9.0	27
36.	4, 3, 7, 3, 4	4.2	35	5, 7, 5, 7, 16	8.0	51
37.	60, 36, 20, 31, 33	36.0	37	5, 7, 7, 9, 3	6.2	33
38.	7, 4, 6, 8, 4	5.8	28	7, 9, 15, 10, 10	10.2	26
39.	10, 12, 12, 13, 14	12.2	11	17, 22, 24, 15, 20	19.6	17
40.	34, 35, 43, 34, 38	36.8	9	68, 42, 40, 57, 60	53.4	20
41.	14, 18, 20, 15, 14	16.2	15	11, 9, 14, 8, 15	11.4	24
	Average	22.6	22		20.2	25

The first series of specimens consisted of 10 stools, on each of which 10 parallel counts were made by each method. The data summarized in table I show that the counts by the two methods are about equally variable, the average of the coefficients of variation being 21 and 20 respectively, for the dilution and direct smear counts. On each stool of a larger series, 5 parallel counts were made by the two methods. In this series, there were 41 stools on which at least 1 egg was found on all 10 slides (5 direct and 5 dilution). The data are summarized in table II. As in the above series, the counts by direct smear show about the same or somewhat less variability than those made by the dilution method; the average of the coefficients of variation being 22 and 25, respectively, for the direct and dilution counts. For 28 series of dilution counts (5–8 counts each) reported by Stoll and Housheer (1926a, b) the average of the coefficients of variation is 23. Thus the two types of counts do not

TABLE 3.—*Mean egg counts and coefficients of variation for three cases examined periodically*

Case	Period of observation in days	No. of stools	No. counts per stool per method	Direct Smear Eggs per slide × 300			Stoll Method Eggs per cc (fsb)		
				Range	Mean	C.V.	Range	Mean	C.V.
I	38	7	1–10	1500–2300	1,929	16	1200–4000	2,651	29
II	14	8	5	2100–6300	4,388	38	1800–10,600	5,280	50
III	9	9	5	750–1800	1,067	26	400–2400	1,338	42

differ widely in degree of variability and, therefore, should be about equally reliable. At least it is evident that coefficients of variation do not indicate less reliability for the direct than for the dilution counts.

Relationship between eggs per slide and eggs per cc of stool.[2]   For the 10 stools on which 10 parallel counts were made, the coefficients of correlation were found to be 0.936 and 0.924, respectively, for the corrected and uncorrected counts in terms of eggs per cc correlated with the direct smear counts in terms of eggs per slide.   For the 41 stools on which 5 parallel counts were made, the corresponding figures were 0.839 and 0.807.   This indicates that the counts by direct smear correlate rather
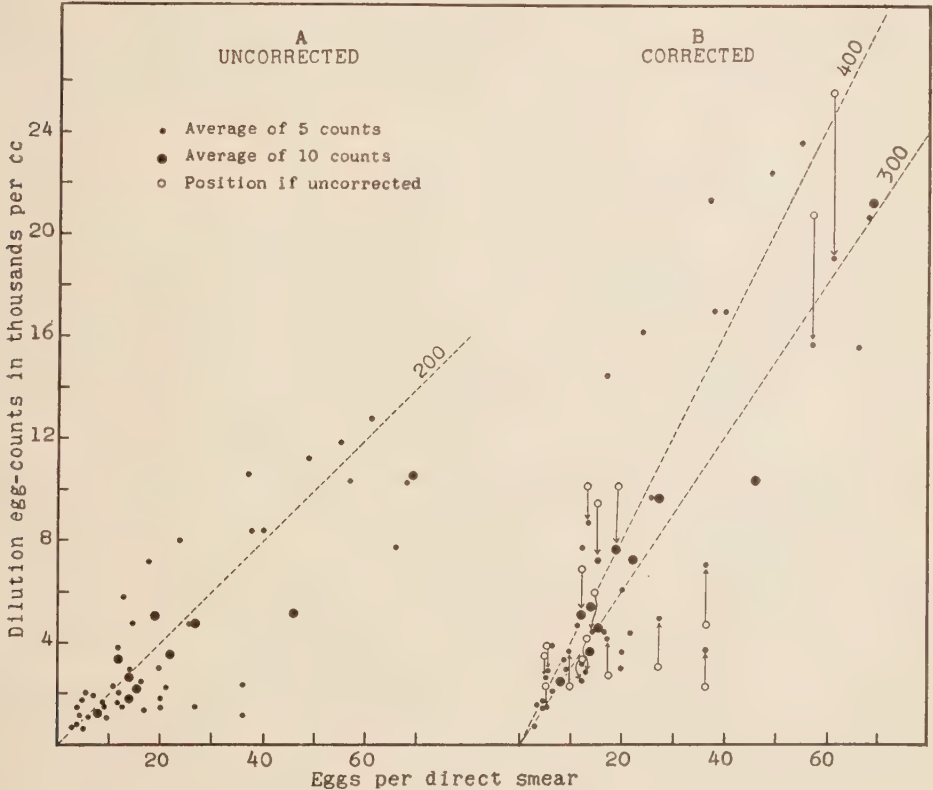

FIG. 2.   Correlation between egg counts by direct smear and counts by dilution.   A. Uncorrected dilution counts show positive correlation.   B. When dilution counts are corrected to the formed stool basis closer correlation is obtained.

closely with dilution counts, both corrected and uncorrected, and that it should be possible to determine a factor for converting direct smear counts to eggs per cc of feces.   However, it is also indicated by the slight differences in the coefficients of

---

[2] To follow the presentation of this section, it will be necessary to keep in mind the essential features of the dilution egg-count method.   A 4 cc sample of feces is measured by displacement in a 60 cc flask containing 56 cc of N/10 NaOH; all of the eggs in a 0.075 cc sample are counted, and the number of eggs is multiplied by 200   ($4/60 \times 75/1000 = 1/200$)   to give eggs per cc of stool. Counts are converted to eggs per cc of formed stool by using the following consistency factors: Formed, 1; mushy-formed, 1.5; mushy, 2; mushy-diarrheic, 3; and diarrheic stools, 4.   Thus a count of 10 eggs in the aliquot of a mushy stool would give an uncorrected count of 2,000 eggs per cc and a corrected count of 4,000 eggs per cc.

correlation for the corrected and uncorrected counts that the clear interpretation of such data requires further study of the question of stool consistency.

Of the 51 stools included in the above analysis, 19 were of consistencies other than mushy (factor 2). Two were factor 1, 12 were factor 1.5, and 5 were factor 3. Graph A of figure 2 shows the dilution counts on all 51 stools plotted against the direct smear counts. Locating each of the factor 1.5 and factor 3 stools on graph B of figure 2 revealed the significant fact that in no instance did the correction increase the spread in the plotted points, and in all instances where the points on graph A (uncorrected) were at or near the margin of the spread, correction produced a closer grouping of the counts. This indicates that counts by direct smear correspond with dilution counts corrected to the formed stool basis (fsb) rather than with the actual number of eggs present per cc or per gram of stool, and that the factor for converting eggs per direct smear to eggs per cc (fsb) is between 300 and 400—probably nearer the former.

Since the direct smears are made to a standard density and, therefore, contain a standard quantity of light reducing matter rather than a standard quantity of feces (particulate matter plus a variable amount of moisure), it is to be expected that they correspond more with corrected than with uncorrected dilution counts. That this is true, and that lack of closer correlation in the two types of egg counts is due very largely to the small number of subjective and discrete classifications of stool consistencies is suggested by the eggs-per-slide relationship with reference to the density of the dilution suspensions and the stool consistency. In general, formed stools produce a large quantity of sediment in the dilution flasks, and the 0.075 cc samples of the suspension from these flasks give relatively low foot-candle readings when measured by the meter used for standardizing the direct smears. That is, such drops of suspension, bearing a great deal of opaque particulate matter, reduce the transmitted light from 20 down to 10 foot-candles or less, while the very fluid stools generally reduce the light only slightly, sometimes as little as 1 or 2 foot-candles, from 20 down to 19 or from 20 down to 18. For convenience, the density of dilution suspensions as shown by light meter readings has been recorded as 20/x. The above examples would be referred to as 20/19 and 20/18.

When stools of each consistency type were grouped according to density, it was found that there was considerable range in each class, especially in the factor 2 class (figure 3). The mean densities, however, in spite of small numbers in the end classes, indicated a linear type of distribution and a positive correlation between the consistency of the stool and the density of the dilution suspension.

Because the factor 2 class was both the largest and had the greatest range in density, this group was analysed with reference to egg counts by direct smear. It was found that if dilution egg counts were plotted against direct smear counts as three separate classes, (1) a mid-class consisting of counts on stools having a density of 20/12 to 20/15, (2) a less dense group having densities above 20/15, and (3) a more dense group having density readings of less than 20/12, it could be clearly shown that each of these three classes of stools formed a separate curve. There was, of course, considerable overlapping between the mid-class and both of the end classes but there was no overlap in the distribution of the two end classes. It was evident that closer correlation with direct smear counts could be obtained by

subdividing the "factor 2" class of stools on the basis of density of the dilution suspension.

It may be seen by inspection of the data in tables I and II that egg counts per direct smear may be markedly higher or lower than counts per preparation (not per cc) by the dilution method. In well-formed concentrated stools it would be expected that the direct smear counts (i.e., eggs per slide) would be lower, while in diarrheic stools they would be higher than corresponding counts on the dilution
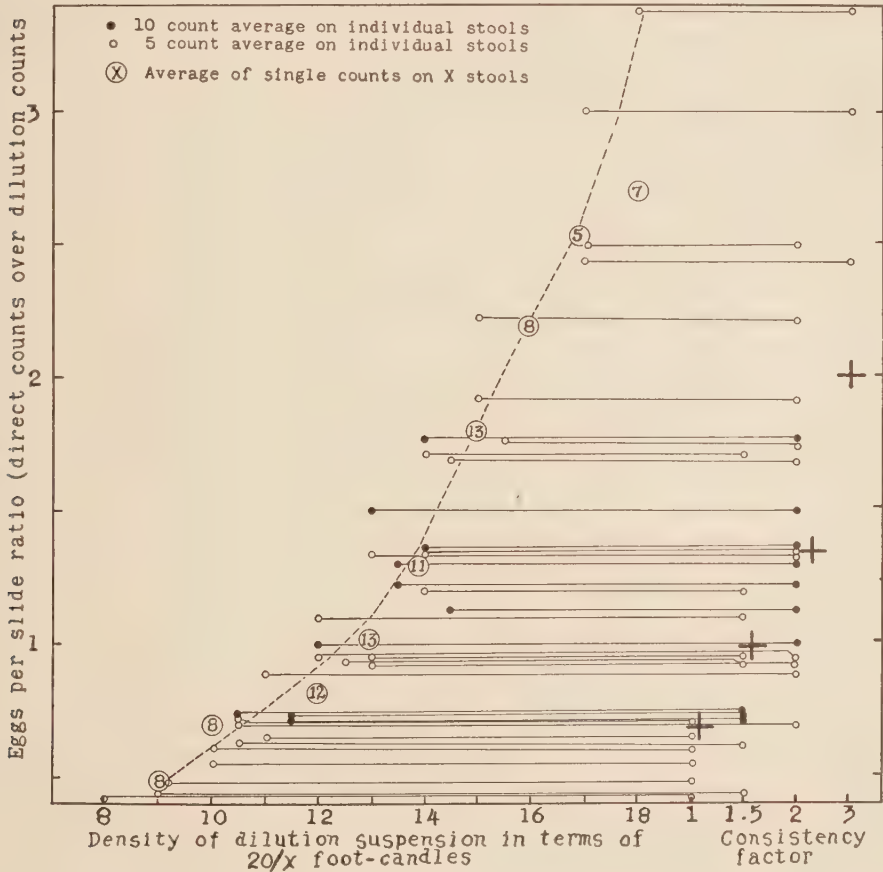

Fig. 3. The relationship between the relative number of eggs per slide by the two methods, the density of dilution suspensions, and consistency of the stools. Formed (factor 1) stools give dense dilution suspensions which contain about twice as many eggs per slide as the direct smears. The more diarrheic stools give progressively less dense dilution suspensions which contain proportionately fewer eggs per slide as compared with direct smears. Plus marks, "+", indicate the ideal position for the average stool of each consistency type.

preparations. If, then, direct smear counts, in terms of eggs per slide, are divided by the corresponding counts by the dilution method, ratios expressing the relative number of eggs per slide by the two methods are obtained. For example, if the counts on any given stool are the same by the two methods a ratio of 1/1, or 1.0, would be obtained; 16 eggs by direct smear and 10 by dilution would give a ratio of 1.6/1, or 1.6, etc. These ratios may be seen plotted against the corresponding

20/x densities in figure 3 where a high degree of positive correlation is evident. Stools having approximately equal numbers of eggs per preparation by the two methods all have about the same density of the dilution suspension, namely 20/12 to 20/13. Where the ratio is 1.3:1, the density readings are around 20/14; where it is 2:1, the density readings are around 20/15, etc. It will be noted that the greater the density of the dilution suspension, the higher will be the eggs-per-slide counts by dilution as compared with the counts by direct smear on the same stool. This relationship does not describe a straight line curve, however.

In order to obtain additional indication of the appropriate factor for converting the standard direct smear counts to eggs per cc, these same stools (in figure 3) are identified with their respective consistency classifications. The most direct comparison between direct and dilution counts should be possible in that particular consistency class of stools which comes the nearest, on the average, of giving the same egg counts per preparation by both methods. It can be seen that this is true for the mushy-formed (factor 1.5) group of stools. The median class of mushy-formed stools has a density of about 20/13 and gives eggs per preparation counts equal to the direct smears (eggs per slide ratio of 1). Therefore, since mushy-formed stools have a correction factor of 300 (1.5×200), direct smear counts likewise should be converted to eggs per cc (fsb) by using 300 as a factor. On this basis, the theoretical or ideal positions for all classes have been determined and are shown by the plus sign (+) in figure 3. There can be no doubt that using 300 as a conversion factor for standard smear counts will give acceptable values for the average mushy and mushy-formed stools. Due possibly to peculiarities of the few stools dealt with, the ideal and the actual medians for factor 1 (formed) and factor 3 (mushy-diarrheic) stools do not agree. It appears probable that the Stoll correction factors overcorrect the more formed and undercorrect the less formed stools.

Day-to-day variability in egg counts. Using 300 as a conversion factor for the direct smear counts, two stools collected from several days to a few weeks apart were egg-counted on 19 individuals, and 7–9 periodic counts on 3 other cases were obtained for a comparative study of day-to-day variability and level of infection. In case I the counts were fairly uniform by both methods even though only single counts were made on 4 of the 7 stools. In case III, the counts by direct smear were much less variable than dilution counts. No one single stool, egg-counted by the direct smear method, would have been misleading with reference to intensity of the infections in cases I and III. Counts on case II were more variable by both methods. Direct smear counts varied between 2,100 and 6,300 while the spread in the dilution counts was somewhat greater, 1,800–10,600. Results are summarized in table III.

Counts on the 19 individuals egg-counted on two separate occasions were about equally consistent by the two methods. In 12 instances the second counts by both methods agreed closely with the first. In 5 instances the second counts increased or decreased in about equal degree, while in one case for each method the second counts were in sharp disagreement with the first.

Relationship between egg counts and hookworm burden. Following treatment with tetrachlorethylene, worm counts were obtained on stools passed during the first 24 hours. Egg counts were repeated 4 to 10 weeks after the treatment. The treated subjects were 7–14 years of age. Treatment results were on the whole

disappointing. Only 4 out of 64 treatments resulted in complete removal of worms, and in all four cases the infections were very light.

Since it was not possible to be sure that all worms removed by the treatment were recovered, it was considered best to select for analysis only the 10 cases which, having had at least 5 pre- and 5 post-treatment egg counts, showed the greatest relative decrease in egg counts per mature female recovered. The results in terms of mature females recovered per egg per standard smear are: 6.3, 6.0, 5.2, 5.0, 4.5, 4.4, 4.0, 3.4, 3.2, and 2.7. The corresponding figures for dilution counts in terms of eggs per cc (fsb) per mature female are: 62, 58, 57, 96, 59, 65, 37, 130, 124, and 119. Since each mature female contributes around 50 eggs per cc of stool (Stoll, 1923b), the last 3 of the above series should be discarded on the assumption that a significant proportion of the worms removed by treatment were not recovered. The range for the remainder is 4.0–6.3 with an average of 5 females. Thus, it may be concluded tentatively that each egg by standard smear count represents roughly 10 mature worms, 5 males and 5 females.

<center>DISCUSSION</center>

If the purpose of egg counting is to estimate the intensity of an infection rather than the actual number of eggs in a given quantity of feces, direct smear counts apparently are as reliable as dilution counts. Now that smears can be made to contain uniform quantities of fecal substances, the most probable source of important error in the smear technic would seem to be in the smallness of the samples taken. However, as compared with the dilution sample actually examined for eggs, the standard smear sample generally is not smaller. In both the 10-count and 5-count series (tables I and II) the quantity of feces examined must have been on the average slightly greater in the smear samples because the average number of eggs counted was somewhat greater. The principal question yet to be answered is whether hookworm eggs are sufficiently unequal or non-random in their distribution in the feces to make 1/200 cc of a diluted sample of mushy-formed (factor 1.5) stool more adequate or reliable than 1/300 cc of the undiluted feces. In each case the fsb corrected estimates are based on about 1/300th cc samples. This and a number of other important questions regarding the individual sources of error will require further study. At present the error in egg counts is known only roughly by comparing one series with another. The theoretical expected total error of estimate such as has been established for blood cell counts (Berkson et al., 1940), has not been determined for the estimation of eggs per unit of feces by any method.

The direct smear technic apparently requires no correction for consistency or size of the stool. On different portions of the same stool, counts average the same regardless of consistency, because the moisture content of a stool does not appreciably influence the quantity of feces (less moisture) in the standard direct smear. However, the relative size and consistency of the stool may or may not be due to its moisture content. In cases where moisture is not the principal diluent of the feces, the increased mass may be due to elements that do not appreciably influence the amount of feces that go into the standard direct smear. Bulky stools do, however, contain variable amounts of diluents (seeds, fiber, etc.) that are included in the 4 cc samples used for dilution counts. Since all gross elements are removed from the direct smear, they contain for the most part only eggs and what

might be called pure feces, consisting of such elements as secretions of the alimentary tract and its appendages, the intestinal flora, and the various substances which contribute to the colloidal mass that give it characteristic form—all of which are more or less constant from day to day in the normal individual. The bulk and consistency of stools will be altered by changes in diet to a much greater extent than will the "pure feces" element.

Conflicting views regarding the usefulness of corrected, as compared with uncorrected egg-count data (Scott and Headlee, 1938) may be resolved on the pure feces concept. If data are based on counts made on individuals or on population groups in which the consistency of the stool is primarily altered by moisture (low residue diet), Stoll's consistency factors would correct counts to less variable data; whereas, if stools are bulky and unformed because of increased proportion of gross elements that fail to give them "form" (high in diet residue, relatively low in colloidal mass), the same factors would undercorrect the more formed, and overcorrect the less formed stools.

Ideally, for the direct smear counts, there should be some correction made for color of the feces. Although the light which passes through the fecal smear onto the meter's cell is reduced primarily by diffusion, there is also a certain amount of reduction by absorption. Smears from the darker stools will, of course, absorb more light than those from the paler ones. Since the film is thin, the absorption factor probably can be regarded as negligible in most cases. However, it should be taken into account in such extreme cases as black stools resulting from iron therapy, or the clay-colored stools resulting from liver dysfunction.

The question is likely to arise as to whether reliable egg counts can be obtained from ordinary unstandardized direct smears. Experienced technicians generally make fecal smears of such density as will easily allow reliable identification of light, moderate, and heavy infections, but it is not possible to interpret counts from such smears in terms of eggs per cc. In general, reliable conclusions can be drawn from direct-smear counts made by different individuals only if the smears have been made to a standard density. When an absolutely uniform calibration standard has been developed, the photoelectric foot-candle meter should become a practical, easily adaptable, and inexpensive instrument for that purpose.

SUMMARY

1. The direct fecal smear can be standardized by using a photoelectric type of light meter to measure its turbidity.

2. Multiple counts on individual stools do not vary more by the standard direct smear than by the Stoll dilution method.

3. There is close positive correlation between standard direct smear and dilution counts, the correlation being closer when dilution counts are corrected to the formed stool basis.

4. Standard direct smear counts can be interpreted roughly in terms of eggs per cc (formed stool basis) by using a conversion factor of 300.

5. Day-to-day egg counts on the same individual are as uniform by standard direct smear as by dilution methods.

6. Standard direct smear egg counts can be interpreted in terms of worm burden, each egg representing roughly 10 mature hookworms (*Necator americanus*).

References

BERKSON, J., MAGATH, T. B., AND HURN, M. 1940 The error of estimate of the blood cell count as made with the hemocytometer. Am. J. Physiol., **128**: 309–323.

CORT, W. W. AND PAYNE, G. C. 1922 Investigations on the control of hookworm disease. VI. A study of the effect of hookworm control measures on soil pollution and infestation in a sugar estate. Am. J. Hyg., **2**: 107–148.

HAUSHEER, W. C. AND HERRICK, C. A. 1926 The place of the smear in hookworm diagnosis. Am. J. Hyg., **6**: July Suppl., 136–148.

KELLER, A. E. 1934 A comparison of the efficiency of the Stoll egg-counting technique with the simple smear method in the diagnosis of hookworm. Am. J. Hyg., **20**: 307–316.

SCOTT, J. A. AND HEADLEE, W. H. 1938 Studies in Egypt on the correction of helminth egg count data for the size and consistency of stools. Am. J. Hyg., **27**: 176–195.

SMILLIE, W. G. 1921 A comparison of the number of hookworm ova in the stool with the actual number of hookworms harbored by the individual. Am. J. Trop. Med., **1**: 389–395.

SOPER, F. L. 1926 Comparison of Stoll and Lane egg-count methods for the estimation of hookworm infestation. Am. J. Hyg., **6**: July Suppl., 62–102.

STOLL, N. R. 1923a Investigations on the control of hookworm disease. XV. An effective method of counting hookworm eggs in feces. Am. J. Hyg., **3**: 59–70.

————1923b Investigations on the control of hookworm disease. XVIII. On the relation between the number of eggs found in human feces and the number of hookworms in the host. Am. J. Hyg., **3**: 156–179.

STOLL, N. R. AND HAUSHEER, W. C. 1926a Accuracy in the dilution egg counting method. Am. J. Hyg., **6**: March Suppl., 80–133.

————1926b Concerning two options in dilution egg counting: small drop displacement. Am. J. Hyg., **6**: March Suppl., 134–145.

PRESENT STATUS OF TRICHINOSIS IN SANTIAGO, CHILE*

Dr. Amador Neghme †

Trichinosis is endemic in Chile. This is shown by the fact that during the past 10 years small familial epidemics have been reported throughout the province of Santiago, and every year not less than 50 human cases occur in that region. During the year 1944, examinations for trichinae were made of the diaphragm muscle tissue from 296 persons coming to necropsy in several hospitals of Santiago; trichinae were found in 12.5 per cent (Neghme, 1944). In spite of the widespread distribution of the parasite, the mortality rate of clinically recognized trichinosis in Chile is very low and most of the cases are benign. This is illustrated by the following brief summary of an outbreak that occurred during the winter of 1947 in the Military School of Santiago in which 422 of 470 persons studying and working in the Military School were affected. Skin tests and haematological examinations involving the entire group of 470 persons were carried out July 21 and August 8 following the start of the epidemic, and clinical studies were made of the 422 persons affected. Of these, 351 exhibited clinical symptoms of trichinosis; in the case of 71 the disease was subclinical, but in all of the latter group a high eosinophilia was found and skin tests were positive. Of the total number of cadets, 94 per cent were stricken with the disease while among the assistant soldiers and servants 70 per cent were affected. No fatal cases were reported and, in general, the clinical cases were mild (benign). The incubation period in 339 of the clinical cases ranged from 4 to 25 days, the average being 16 days; in 97 per cent the incubation period was less than 22 days. The source of the outbreak in question was ascertained to be imperfectly cooked (fried) sausages served at lunch one day in the dining room of the institution. Investigation disclosed that the pork used by the wholesaler in preparing the sausages had not been subjected to microscopic inspection for trichinae (Neghme et al, 1947).

In addition to the outbreak in question, 157 clinically acute cases of trichinosis were found in several parts of the city during the period between July and October of that year. The cases were distributed throughout the population without predominance of sex or age. Investigations to ascertain the sources of the outbreak showed that from a month before the beginning of the epidemic, many hogs were purchased and slaughtered without microscopic inspection for trichinae. Another factor that is thought to have contributed to the outbreak was a transient shortage of beef which resulted in an increased consumption of pork prior to and during the period in which the outbreak in question occurred.

Investigations have been carried out in the municipal slaughterhouse of the city of Santiago where many thousands of hogs are slaughtered annually, to ascertain the annual incidence of trichinae in swine coming to slaughter in that establishment. As shown by the examination of one or more specimens of diaphragm muscle tissue from each hog, the incidence of trichinae in the animals examined ranged from 0.2 to 0.3

per cent, being highest in hogs raised in garbage lots where rats, corpses of dogs, cats and perhaps pork scraps are abundant in the garbage fed.

Microscopic inspection of slaughtered hogs for trichinae is compulsory in Chile and is routinely carried out by veterinarians. In the past, the examinations have been made, for the most part, on one sample of diaphragm muscle tissue per hog. In spite of regulations governing the post mortem inspection of hogs for trichinae such examinations have not been carried out universally. This is shown by the findings of a survey involving 52 municipal and private slaughter houses in the province of Santiago. In this survey it was found that microscopic inspection for trichinae was being performed as a routine measure in only 5 (9.6 per cent) of the establishments surveyed. In view of this fact there was approved, in August, 1947, a new regulation (see References) governing the post mortem inspection of hogs for trichinae. The provisions of the regulation are briefly as follows: (1) Hogs shall be slaughtered only in slaughterhouses in which microscopic examinations for trichinae are routinely carried out; and (2) Eight specimens of muscle from each hog shall be examined. In addition to these provisions the regulation prohibits the raising of swine in garbage lots and the feeding of uncooked garbage. In connection with enforcement of the latter provision of the regulation, 822 hogs found in one garbage lot in the environs of Santiago were slaughtered and examined for trichinae; 5 per cent of the animals examined harbored the parasite. To obtain information on the source of infection of these hogs with trichinae, 200 rats trapped on the premises were examined; 8 per cent harbored trichinae (Neghme *et al,* in press).

The writer agrees with Gould (1945) that processing all pork to destroy trichinae is the most effective method of controlling trichinosis. Unfortunately such measures are not feasible in Santiago at this time. Consequently, reliance must be placed on the control measures embodied in the new regulation. It is considered that the raising of swine under more hygienic conditions, abolition of the practice of feeding raw garbage, and improvement of the methods of microscopic inspection will help to control the spread of trichinae. Microscopical inspection, especially by the phototrichinoscope, has not been found to be so expensive in Chile as reported for other countries. Measures to control trichinosis by education of the public to adequately cook all pork are being carried out also and, in general, the public is becoming aware of the danger of acquiring trichinosis from consuming pork that is inadequately cooked.

REFERENCES

Diario oficial de Chile. 1947 Regalmento para la profilaxis y prevención de la Triquinosis. No. 20827: 1884–1886, August 14, 1947.
GOULD, S. E. 1945 An effective method for the control of trichinosis in the United States. J. Amer. Med. Assoc., **129**: 1251–1254.
NEGHME, A. 1944 Epidemiología de la Triquinosis. Biologica, **1**: 127–140.
NEGHME, A., HOECKER, G., AND FELNER, E. Triquinosis en las ratas de los basurales. In press.
NEGHME, A., ROMAN, J., FAIGUENBAUM, J., PIZZI, T. *et al*. 1947 Brote epidémico de triquinosis en la escuela militar de Chile. Bol. Of. San. Pan. **26**: 921–935.

RETROFECTION IN OXYURIASIS. A NEWLY DISCOVERED MODE OF INFECTION WITH *ENTEROBIUS VERMICULARIS*

W. Schüffner

Hilversum, Holland

and

N. H. Swellengrebel

Amsterdam, Holland

INTRODUCTION

Until a short time ago the mouth was considered as the only portal of entry of *Enterobius vermicularis* into the body. Although other means of infection have been occasionally considered, no definite conclusions have been reached. Only since the Amsterdam experiment, in which 6 persons out of 8 were successfully infected with dried eggs from room dust, has the air-borne mode of infection *via* the mouth and nose been definitely established (Schüffner and Swellengrebel, 1943, 1944). The important rôle of air-borne eggs for the dissemination of the infection thus became evident.

Children's homes and primary schools have been found to be centers for the dissemination of the eggs in the Netherlands (Swellengrebel and Schüffner, 1946; Schüffner, 1947). The eggs lost from the underwear get into the room dust, as Reardon (1938) and Nolan and Reardon (1939) have shown previously, and may accumulate there in large numbers. In dust that was at most 6 days old, in areas of about 10 square decimeters, up to 300 eggs were found. In rest rooms, where the dust had accumulated over a longer period, up to 5000 eggs were recovered.

Despite these surprising numbers, such dust-borne eggs will reach the human body only in moderate numbers, in contrast to the anus-to-mouth transmission by fingers, by which method 100 or even 1000 eggs may be introduced at one time. Relatively few dust-borne eggs actually will produce infection. Nevertheless, they constitute a continuous menace to many people; and from time to time a person will become infected. In schools it is rare for a child to escape infection and thererafter transmission by the fingers will build up the worm burden.

It may be mentioned that it is incorrect to ascribe permanent stickiness to the egg. Dried eggs can very easily be separated from the substratum; one actually gains the impression that they are especially suited to dissemination by dust.

Depending upon the mode of entry of the eggs, different pictures of the infection result. This is best demonstrated if the results of daily anal egg recoveries are shown in the form of graphs. Such graphs (fig. 1 to 3) have the added advantage of showing the details of the infection in a much more complete way than counting escaped worms, the only method used by previous workers.

Fig. 1-A shows direct transmission by fingers, with its uniform picture of mass production of worms, a condition common in children, and the chronic course of the infection until it is terminated, at least temporarily, by treatment. In schools it is responsible for the heavy contamination of dust. This type is found in adults
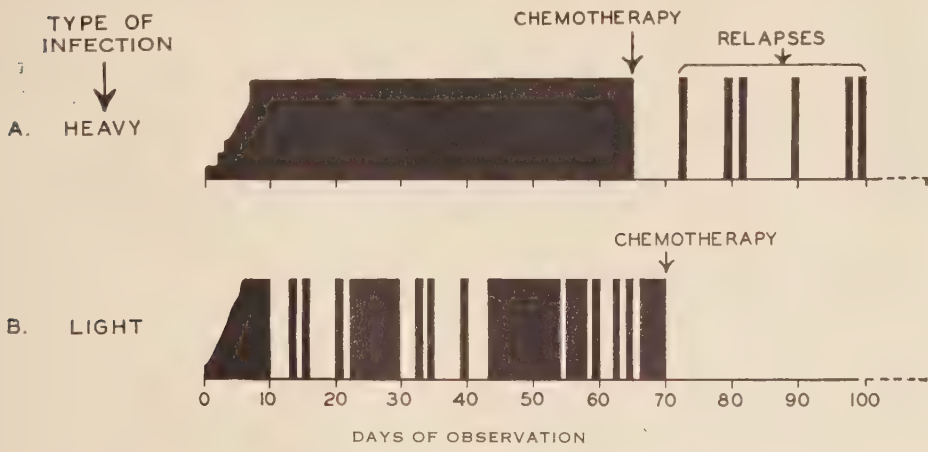
Fig. 1.—A, graph showing the course of typical anus-to-mouth transmission of oxyuriasis by fingers, based on recovery of eggs of *Enterobius vermicularis* from the anal region; B, the same, restricted by mechanical prophylaxis.

only in cases of gross uncleanliness (i.e., mental derangement, poverty, etc.). It chiefly causes autoinfections.

Fig. 1-B shows the same type, the only difference being that the introduction of eggs was restricted by mechanical prophylaxis. Egg-free days and zones appear, indicating that the prophylaxis was partially effective.

Fig. 2 shows the indirect or contact infection which contaminates the fingers and from there reaches food. The number of endangered persons is thereby considerably enlarged, namely through heteroinfections of hitherto worm-free persons. An acute attack occurs during which the worm production first increases and then decreases until the ingested eggs have become exhausted. Self cure occurs in adults; the artificial infections in the Amsterdam experiment, which ended spontaneously, belong to this group. In children, the fingers bring about a continuation of the infection and prevent a self cure. Under normal conditions this type is an exception; the senior writer saw it only once in four years in the members of his household.
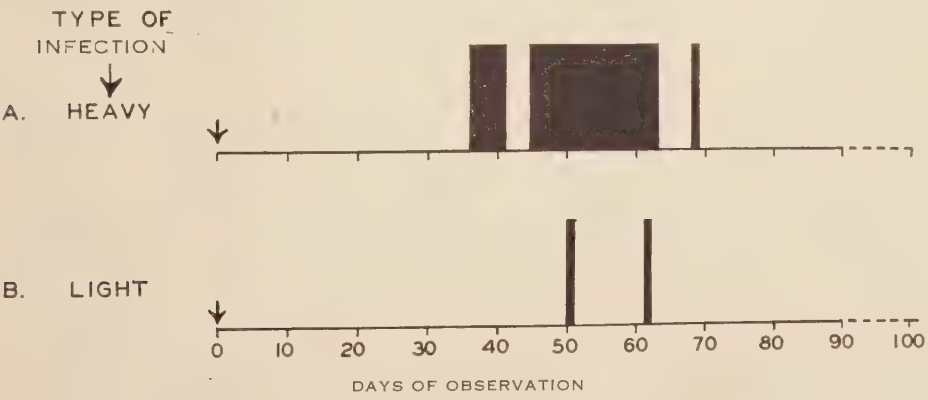

Fig. 2.—Graph showing the course of indirect or contact method of transmission, based on recovery of eggs from the anal region.
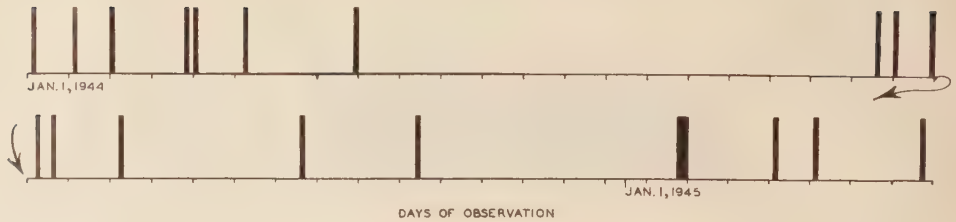
DAYS OF OBSERVATION

Fɪɢ. 3.—Graph showing the course of the dust-borne mode of transmission, based on recovery of eggs from the anal region.

Fig. 3 shows the dust-borne egg infection which is encountered only in persons living in a dust-rich environment, as among school children and school personnel. This is likewise a chronic infection but differs fundamentally in its course from the two types just described. The worms appear sparsely, are irregularly distributed throughout the year, and are usually ephemeral. Series of successive egg-positive days, as seen in the other types, do not occur or are the exception. Dust will therefore hardly ever give rise to a heavy individual infection. In many cases it will escape attention unless daily egg search is practiced. Depending upon external conditions which may bring a person into a dust-borne egg-rich atmosphere or may remove him from there, as on holidays, sickness, etc., the intervals may be practically of unlimited duration. This type serves better than any other for the widespread distribution of the infection.

A fourth type, which has hitherto been unknown and has been confused with the finger mode of infection, is due to larvae which one can occasionally find hatched and crawling on the anus. This curious behavior which was generally regarded as precocious and therefore useless larval development, suggested to Langhans (1926) twenty-two years ago and recently to the senior writer the idea that this might be a mechanism allowing the larvae to migrate upwards from the anus into the bowel.

PRESENTATION OF DATA

The decisive experiment was carried out with the coöperation of a colleague who will be designated as Doctor B. He undertook to carry out on himself the necessary daily egg search over a period of two years. He died early in 1947 and therefore had no opportunity to see the outcome of his unique work.
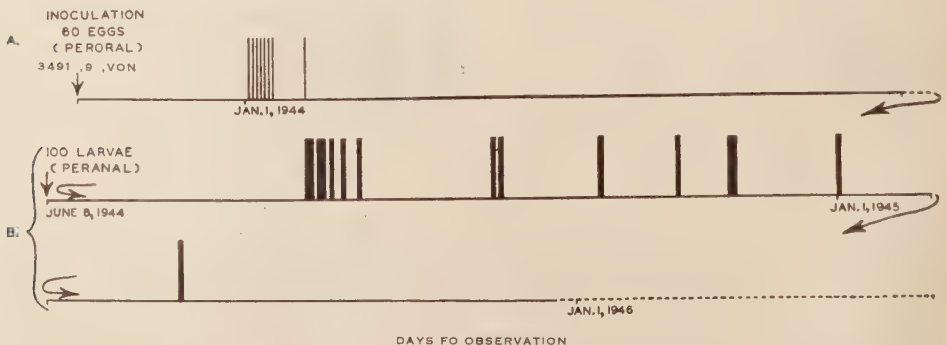

DAYS FO OBSERVATION

Fɪɢ. 4.—Graph showing the course of Doctor B's earlier experimental infections (A) and experimental retrofection (B).

On two occasions Doctor B. underwent experiments with pinworms. In 1943 he participated in the Amsterdam experiment. Patency began in his case on the 55th day after ingestion of about 60 dust-borne eggs and terminated on the 69th day (Fig. 4-A; Fig. 6, 3). Before and after the attack the egg-search remained negative (for a total period of about 10 months) and consequently indicated that no danger of inter-current oxyuriasis existed, a guarantee which was missing in most earlier experiments.

On the 8th of June, 1944, the senior writer applied about 100 free larvae, hatched in artificial gastric juice, to the sphincter portion of Doctor B's anus. August 23, that is, on the 76th day, the onset began; it terminated on the 88th day. To judge from the egg deposits, females had oviposited on seven out of these thirteen days. Then a long interval occurred. The result of this experiment with its well-defined brief period of patency paralleled closely the earlier oral infection but the prepatent period was longer than in the latter (37 to 57 days). The onset, (Fig. 4-B), how-
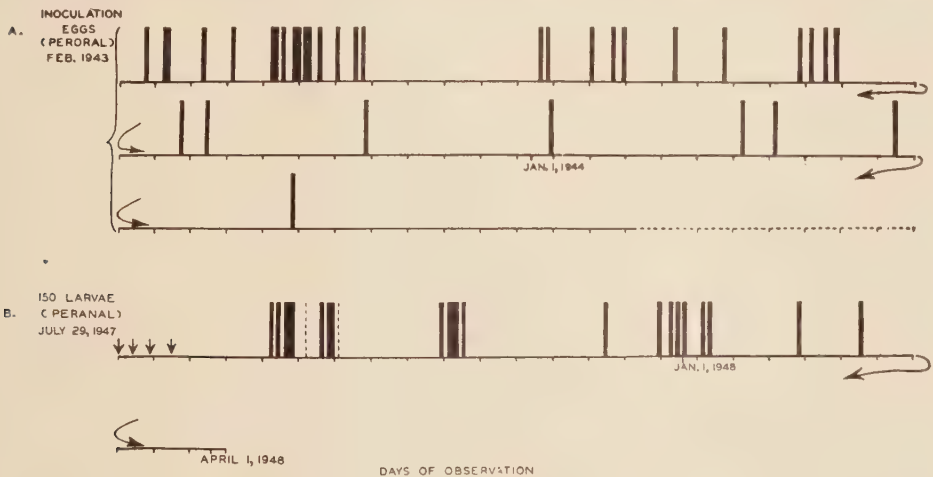
Fig. 5.—Graph showing the course of the senior writer's earlier casual infection (A) and experimental retrofection (B).

ever, was still within the average values known for the life span of the female worms (37–93 days, fixed by the Amsterdam experiment, Fig. 6).

A single experiment was obviously not decisive; a second was necessary and this one was carried out by the senior writer on himself a short time ago. It should be mentioned that he knowingly harbored pinworms only during 1943–44 (Fig.5-A). From September, 1944 on he was again worm-free. Since he reacted acutely to the stimulus of crawling worms, it was not necessary, as in the case of Doctor B, to carry out daily egg search. These were necessary only when indicated by a stimulus in the anal region. Tickling sensation can, however, also be due to other causes. Consequently in the course of three years many negative egg searches were made to be certain that no worm was overlooked.

The infection itself differed from Doctor B's in that the peranal inoculation had to be repeated several times in order to reach sufficient numbers to produce a demonstrable infection. This was due to a casual lack of suitable eggs.

The experiment (see Fig. 5-B) began July 29, 1947 and was still under observation a year later.   A total of about 150 larvae were administered; from then on daily search for eggs was carried out.   The first female appeared September 8, 1947; the prepatent period (43 days) corresponded exactly to the expected values.

The primary attack in this second case (19 days) lasted somewhat longer than in the case of Doctor B.   This may be due to the larger and somewhat protracted inocula of larvae, but the difference has no sigificance in view of the great variability seen in peroral infections with eggs: during nine such infections, eight with dust-borne eggs and one with eggs directly from the anus, the infectibility was found to vary between 0 and 40 percent (Fig. 6).
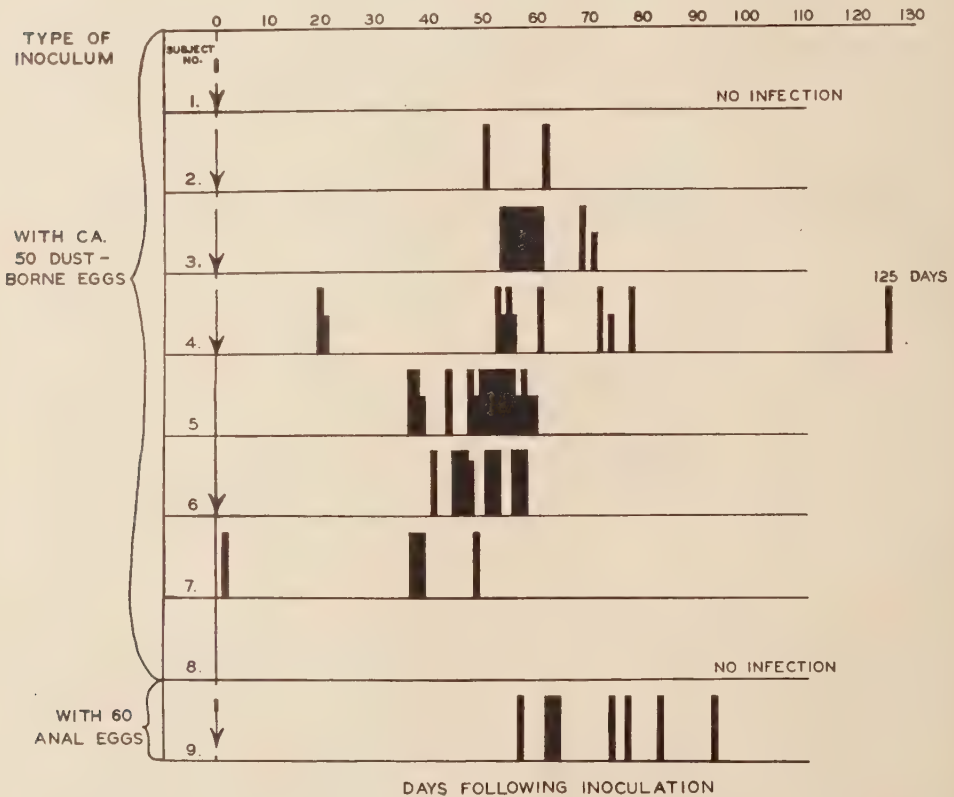
Fig. 6.—Graph showing the course of peroral infection with oxyuriasis.   In nos. 1–8 eggs from dust were employed; in no. 9 eggs from the anus were utilized.

During 1948 a third peranal infection was undertaken.   The subject, J. B., a student aged 22 years, had been negative for oxyuriasis as determined by perianal swab examination from November, 1947 until April 9, 1948, when he was inoculated with about 50 larvae.   Following an incubation of 76 days there was an initial attack which lasted 26 days.   This test, which closely resembles that of Dr. B. (Fig. 4-B), is still under observation, but it tends to remove any lingering doubt of the actual existence of a retrograde mode of infection and its protracted prepatent period.

Since both sexes are about equally common in eggs (Heller, 1878), it is possible to calculate approximately how many larvae reached their goal.   Of the 50 possible

females in the case of Doctor B, seven developed and of the approximately 75 in the senior writer's case 8 did so, that is, 14 and 11 percent respectively. This result compares favorably with the oral route of infection.

The retrograde mode of infection or, more briefly *"retrofection"*, which appears so difficult for a small larva, may be more easily visualized by the following calculation. As measured under the microscope, the 140 μ-long larva travels about 100 μ per second, that is, 6 mm. per minute. The 1200 mm.-distant cecum could therefore be reached in 3 to 4 hours. Actually the larva may require a multiple of that time. Even in this case the travel time should have no more influence on the prepatent period than the much longer route along the intestinal mucosa required in cases of oral inoculation.

The prolonged egg search in the case of Doctor B brought another, entirely unexpected confirmation. On the 47th day after the onset of patency, a worm again appeared and this one-day-long positive record was followed by 23 additional ones which were irregularly distributed over the year. A total of 24 recidives* with probably not more than 27 females occurred.**

This reappearance of worms did not come up to our expectation and was at first a disappointment. It looked as if Doctor B was subject to intercurrent reinfection, as unbelievable as this appeared after the smooth course of the earlier oral infection. The experiment appeared without value. Only after doctor B's death, a renewed analysis showed that no reinfections could be involved. And this became certain after the results of the senior writer's own experiment were known.

In order to evaluate correctly the curious course of the infection the following questions required consideration. The small-scale production of eggs and the one-day periods of patency, of which there were 21, provided a puzzling problem. The most probable explanation was a dust-borne egg infection which might produce such a vacant picture. But there was no indication whatever of a dust-borne egg exposure in Doctor B's surroundings. This could not have been responsible. Moreover, contact infection, which is rare under ordinary circumstances and which produces only attacks, could certainly not be used to explain the above chronic condition. Finger infection, at least unusual in itself in adults, is unlikely because Doctor B took special precautions to avoid it. He had also recovered from his oral infection, just as the other 6 positive subjects in the Amsterdam experiment had, without having experienced a single subsequent reinfection. Under these circumstances it is difficult to visualize how, after the peranal infection, the fingers should have caused reinfection time and again. This would presuppose a change of personal hygiene for which all indications were lacking. If one considers furthermore that the graph in no way corresponds to those resulting from finger infections (*vide* fig. 1), it follows that not a single one of the known ways satisfactorily explained the course of the infection in Doctor B. One was dealing with a special type which the writers have designated as retrofection.

---

* *Reinfection* is considered as the return of an infection after the eggs have left the anus before reaching a host. *Relapse* is defined as a reactivation of the infection following inadequate chemotherapy. In order to be detected this must occur before the end of the incubation period of a "new infection". In a *recidive*, on the contrary, the egg remains in place; the larva hatches and does everything itself, i.e., it exclusively produced autoinfection.

** The assumption of this small number is due on the one hand to the "empty" appearance of the graphs (Figs. 4-B and 5-B), and on the other hand to the writers' experience that in such cases invariably only a single worm was captured and that thereafter the tickling ceased.

Retrospectively a second example of this same type has been found in reviewing the senior writer's casual infection during the years 1943–44. The course of this infection can only be explained by retrofection. The graph (Fig. 5-A) was begun only after the primary attack had subsided; the beginning is therefore lacking, it starts with the recidives. These lie first very close together, but then the course is characterized, just as in Doctor B's case, through one-day-long irregular patencies. Yet in the second half of the course the appearance changed fundamentally. The intervals became much longer (40 to 56 days), the periods corresponding to the life span of the females. It would appear then that in the earlier phases several generations overlapped, while later on a single generation was responsible for maintaining the continuity. Thus, a regularity appears that is rather surprising.

In the case of Doctor B the long interval is found only at the beginning and the end of his chart; it occurs each time only once; no periodicity had developed. The senior writer's own experiment, on the contrary (Fig. 5-B), which is still in progress at the beginning of 1949, changed immediately over into a monocyclic type. One recidive occurred after 47 days, the other after 46 days. Although close agreement is most probably due to chance, this periodicity is reminiscent of the fact that 25 years ago Heubner (1922) found in his own infection, which persisted over a period of four years, a definite rhythm of 40 to 50 days. That makes it very likely that Heubner's infection was also due to retrofection. In two of the cases described in this paper the retrofection came spontaneously to an end, (Figs. 4-B and 5-A).

DICUSSION

The actual proofs for the retrofection have been adduced from two types of evidence, on the one hand the sequels of an acute attack and on the other hand the three parallel experiments. There is consequently duplicate proof for an occurrence that in the beginning appeared quite unlikely. Thus, it is appropriate to discuss briefly what is known and what is not known of retrofection.

Of special interest is the question, why no retrofection occurs in one individual (*vide* the Amsterdam experiment) while it does so in others. Even in the same person conditions may change, as is shown in Doctor B's infections. Likewise, in the senior writer's case of peranal infection retrofection developed. Whether the peranal manipulations acted provocatively must be decided by further experimentation.

Other conditions to which retrofection is subject are better known. The intervals between patencies, which may be reduced to a single day, can not exceed certain limits, since one generation—with a lifetime between 37 and 93 days—must follow another in succession. In the senior writer's own infection intervals of 52 and 55 days were observed. Possibly in other cases intervals may last longer, but not longer than the experimentally determined longevity of 93 days according to the Amsterdam Experiment (Fig. 6), quite recently prolonged to 101 days, according to the recent experiment J. B.

By way of contrast dust-borne infection has no upper limit: this has already been emphasized as a fundamental difference.

In so far as the age factor is concerned, age as such does not protect one from retrofection. The aged Heubner was subject to retrofection, as was Doctor B

who was 66 years old and the senior writer, 76 years old in the earlier experiment and now 81 years old. This provides no suggestion of immunity.

In children retrofection probably occurs but it is difficult to demonstrate. The test on volunteer J. B., aged 22 years, proves susceptibility of young adults to this mode of inoculation. The finger mode of infection, with its heavy introduction of masses of eggs, will hide any small-scale production due to retrofection. But one should consider this possibility in cases of exceptionally refractory behavior of an infection.

It should be mentioned that it is by no means certain that in all cases of retrofection only small numbers of worms are produced. The beginning of the senior writer's observations (Fig. 5-B) with its denser picture of infection, proves the contrary. This mode of infection opens a new field for study. One should perhaps think of retrofection in every case of chronic oxyuriasis in adults unless gross uncleanliness is present. This is important for therapeutic considerations. The typical picture as it occurred in Doctor B, or the occurrence of a sequence as in the senior writer's case, makes the diagnosis easy, now that retrofection is demonstrated. The writers know of three other persons who suffered for years from pinworms and in whom one had to assume, in the light of information available at the time, some defect in personal cleanliness. The present investigation frees them from this ungrounded suspicion.

It will now be the task of the family physician to investigate to what extent retrofection is common in adults. Once recognized, termination of the infection can be achieved without drug therapy. Since it is known that these infections can end spontaneously, mechanical prophylaxis must be employed to achieve the goal of interrupting the chains of recidives. This is done by removing, for a certain period of days, the eggs from the anal region before the larvae have a chance to hatch, that is, every 6 hours, the time required for the enclosed embryos to mature in air.

The investigation which the writers have been conducting during the last five years has now come to an end. Much remains to be done, but the fundamental questions are answered. In particular, the refractoriness of some cases of pinworm infection is no longer mysterious.

Transmission from anus to mouth or indirectly by contaminating food with the fingers has hitherto been considered the mechanism responsible for the perpetuation of infection in chronic oxyuriasis. Recently the experiments carried out in Amsterdam by Swellengrebel and Schüffner demonstrated the importance of the air-borne route as a means of distribution of the infection.

In the present paper another mode of infection is described, one which can be taken by larvae hatched in the anal region. This is the retrograde way, or briefly "retrofection", and has been proven by three separate experiments. In adults a special symptom complex is produced in retrofection, which heretofore was confused with the finger type of infection. Its nature was first recognized during an experiment on Doctor B. Its occurrence in children is still unrecognized.

Retrofection becomes especially apparent if the sequence described by Heubner (1922) develops. It does so when the recidives are brought about by monocycles. Two self-observations serve to illustrate this point.

SUMMARY

1. Previous studies on the epidemiology of *Enterobius vermicularis* have demonstrated that infection develops as a result of finger transmission of the eggs from anus to mouth, from indirect contamination of fingers in contact with objects on which eggs have been deposited and due to air-borne eggs getting into the mouth. Mass production of worms is due primarily to the first method. A more widespread but less intense infection is caused by indirect contamination, while chronic light infection results from air-borne eggs.

2. A fourth mode of inoculation, namely retrograde migration of larvae hatched in the anal area, is demonstrated in experiments on three human volunteers. This method, designated as *retrofection,* explains peculiarities in certain cases not accounted for by the other three modes of infection.

REFERENCES

HELLER, A. 1878 In von Ziemssen's Handbuch der speciellen Pathologie und Therapie. Bd. VIII, 2te Hlfte, Leipzig.

HEUBNER, O. 1922 Oxyuriasis. Jahrb. Kinderh., **98**: 1–21.

LANGHANS, G. L. 1926 Zur Biologie des *Oxyuris vermicularis*. Arch. Kinderh., **77**: 27–38.

NOLAN, M. O., AND REARDON, L. 1939 Studies on Oxyuriasis. XX. The distribution of the ova of *Enterobius vermicularis* in household dust. J. Parasit., **25**: 173–177.

REARDON, L. 1938 Studies on Oxyuriasis; number of eggs produced by pinworm, *Enterobius vermicularis,* and its bearing on infection. Pub. Health Repts., **53**: 978–984.

SWELLENGREBEL, N. H., AND SCHÜFFNER, W. 1946 Besmettingsproeven met oxyuriseiern uit kamerstof. Nederl. Tijdschr. v. Geneesk., **90**: 762–764.

SCHÜFFNER, W. 1947 Experimentelle Infektionen mit Staubeiern von *Oxyuris* (*Enterobius*) *vermicularis.* Zentr. Bakt., Parasit. Infektionskr., I Abt. Orig., **152**: 67–73.

SCHÜFFNER, W., AND SWELLENGREBEL, N. H. 1943 Eine zweizeitige Methode zum Nachweis von Oxyuris-Eiern. Ihre Leistung gegenüber dem amerikanischen NIH-Wischer. Zentr. Bakt., Parasit. Infektionskr., I Abt. Orig., **151**: 71–80..

———1944 Der Nachweis von Oxyuren-Eiern am After, im Nagelschmutz und im Zimmerstaub. Idem., **151**: 114–120.

THE MOLLUSCAN INTERMEDIATE HOST AND SCHISTOSOMIASIS JAPONICA. I. OBSERVATIONS ON THE CONDITIONS GOVERNING THE HATCHING OF THE EGGS OF *SCHISTOSOMA JAPONICUM**

JAMES W. INGALLS, JR.,[1] GEORGE W. HUNTER, III,[2] DONALD B. MCMULLEN,[3]
AND PRESTON M. BAUMAN[4]

INTRODUCTION

Throughout the period when the Commission on Schistosomiasis was in the Philippine Islands, all stages in the life cycle of *Schistosoma japonicum* were maintained in the laboratory and considerable time was devoted in attempts to develop the most efficient method of maintaining this life cycle. One phase of this work included observations on the effect of environmental conditions on the hatching of eggs obtained from stool samples.

This was a practical problem related to the study of infections in the molluscan intermediate host. For the study of controlled infections in the snail, it was convenient to have large numbers of miracidia, and to be able to predict the time at which they would be available.

The work was done without prior recourse to the literature, little of which was available in the area. When results reported here concur with other published statements, the confirmatory evidence bears significant value since opinions were not prejudiced.

For comparative reference, the results obtained by some other workers in this field are cited here. In discussing the conditions which are important determinants in hatching after the miracidium is matured and ready to take up a free-living existence, Faust and Meleney (1924) named hypotonicity of the solution, suitable water temperature and degree of active acidity of the medium. They observed hatching over a temperature range of from 8° C. to 37° C., while Miyagawa (1916) had previously given 25° C. to 30° C. as the temperature range most suitable for hatching. Faust and Meleney considered the most important single factor to be the cleanness of the fecal suspension obtained by repeated washings, which reduced the acidity of the water by removing bacteria and yeasts from the stool material. Magath and Mathieson (1946) stressed the importance of having the water of the suspension clean, or at least well oxygenated. They found that water temperatures of 28° C. or 29° C. gave very good results, but they did not obtain hatching when water at 37° C. was used.

Sixty experiments were conducted on Leyte, P. I., during May, June and July of 1945, and an independent series of experiments was carried out by one of us

(GWH) on Mindoro during June of that year. The experimental work performed by the workers on Leyte included investigations into the effect on hatching rates under the following variables: (1) Intensity of illumination; (2) period of day at which eggs were set out to hatch; (3) agitation of fecal matter in the suspension; (4) osmotic stimuli; (5) aeration of the water; (6) age of the stool; (7) water temperature fluctuations and variations; (8) degree of washing of the stool, and (9) pH of the water. A study was made of the effect of varying each of these while all other conditions were constant. The observations made on Mindoro were concerned with the relation of the pH of the water to the degree of miracidial hatching.

MATERIALS AND METHODS

The stools used were collected from six native dogs and a native pig, all having natural infections. For each experiment, the stool from a single animal was utilized. Each day, for sixty consecutive days, two to eight parallel procedures were set up so that only a single condition of handling the fecal material varied.

To make counts of the numbers of hatched miracidia it was necessary to devise a method for concentrating them. The flask used by McMullen and Beaver (1945) in recovering the miracidia of bird schistosomes, did not give good concentration of the miracidia of *S. japonicum*. In experiments with this flask about half of the miracidia appeared in the side arm and the remainder were in the upper water in the neck of the flask. Since the miracidia of *S. japonicum* did not show a marked positive phototropism, advantage was taken of their negative geotropism. Because the miracidia are negatively geotropic, the best method for concentrating those that hatched was to have a small surface area as compared to the volume of water in which hatching was taking place. Excellent results were obtained by fitting a 250 cc or 500 cc Erlenmeyer flask, as subsequently indicated by Stunkard (1946), with a conically bored rubber stopper holding a vertical glass tube. The bottom of the rubber stopper was so cut as to preserve a smooth contour from the wall of the flask to the bottom of the glass tube. The glass tube was made about four inches long and the water level brought up to about one-half inch of the top of this tube. Miracidia would collect towards the top of the water in the glass tube and could be observed by the naked eye or with the aid of a hand lens. When counts of miracidia were to be made, the water in the collecting tube was pipetted into shell vials and these were observed under a dissecting microscope. Complete records were kept of each experiment.

RESULTS

When raw stools were kept over 40 hours at room temperatures fluctuating from 25° C. to 32° C. most of the eggs died. Satisfactory results in hatching miracidia were obtained from stools 18 to 24 hours after they had been passed, but best yields came from feces utilized immediately after being passed. Normal room temperatures referred to above were found most suitable for hatching purposes. Refrigeration almost completely inhibited the hatching process as did temperatures at or above that of the human body. High temperatures, such as 45° C., rapidly killed the miracidia.

Some of the conditions studied were varied without any apparent effect upon the rate of hatching. Variations in intensity of light over the flasks had no apparent effect. The range tested extended from direct sunlight and brilliant artificial il-

lumination to complete darkness. No diurnal or nocturnal cycle in hatching frequency was observed. Agitation of the fecal material, taken as a single factor, did not have any influence on the rate of hatching. The osmotic action exerted by normal saline or 0.5% sodium sulphate solution for one-half hour, followed by washing in fresh water, did not increase the rate of hatching; rather, such exposure was found to decrease hatching percentages.

The two most important factors determining the percentage of hatching were the amount of cleansing of the fecal sediment by repeated washings and the pH of the water used during sedimentation and in the hatching flask. Simple aeration of water was attempted by decanting water from the hatching flask, pouring it in a thin stream between two vessels forty successive times, and then replacing it over the sediment. This was not as effective as sedimentation with fresh water.

In the experiments conducted on Leyte it was noted that there was very limited hatching in natural waters with a pH below 6.6, and that the hatching rate increased to a maximum at a little above pH 7.6. The use of water containing a natural acidic component during the preliminary washings prevented loss of miracidia during this procedure but did not reduce the final total yield. Very heavy yields of miracidia were obtained when a stool was sedimented at room temperature in an

TABLE 1.—*A Statistical Analysis of Experiments Showing the Influence of pH upon the Hatching Rate of* Schistosoma japonicum *Eggs.*[1]

pH range of water	Number of tests	Total number of eggs used	Number of eggs hatched	Percent hatched
6.4–6.8	11	63	10	15.9
6.9–7.1	11	108	48	44.4
7.2–7.6	18	61	43	70.5

[1] P for difference between 15.9 and 44.4 = .001
P for difference between 44.4 and 70.5 = .0005.

acid water (fresh rain water) with a pH of 6.2 to 6.6 and the sediment was then placed in many times its own volume of a natural alkaline water with a pH of 7.6 to 7.8. After the hatching of miracidia had practically ceased, a second and even a third heavy yield were obtained following cleaning of the fecal material by repeated re-sedimentation in acid water and then the addition of alkaline water (pH 7.6 to 7.8) to the flasks.

The alkaline water used was obtained from the Tanauan River, at the same location where Bauman, Bennett and Ingalls (1948) procured water which gave the best results in securing maximum cercarial release from the snails. Through the courtesy of Dr. F. J. Brady, then Acting Chief, Zoology Laboratory, National Institute of Health, a biological analysis of the unfiltered water from the Tanauan River was conducted by Miss Mary Louise Steinle of the Zoology Laboratory and a chemical analysis of the filtered water by Dr. Elias Elvove of the Chemistry Laboratory. The chemical analysis revealed an ionic content of this water which may have been influenced by its proximity to the sea, since the sodium-calcium residue ratio was 185.6/27.2. Dr. Elvove obtained a hydrogen-ion concentration value of pH 7.7.

A statistical analysis of the observations made on Mindoro is presented in Table 1. In the experiment, counted numbers of eggs were placed in embryological watch glasses in water of a known pH. For each test five to eight eggs, each containing a viable miracidium, were used. Examinations were made with a dissecting binocular

microscope over a 48-hour period to determine the numbers which had hatched. Waters with eleven different pH values were used in this series of tests. An original chart prepared in the field by Col. Hunter showed a tendency towards increase in the percentage of eggs which hatched to be correlated with the increase of values for pH. Through the courtesy of Dr. Ernest Carroll Faust a statistical analysis of the original chart was prepared by Dr. Huldah Bancroft, Associate Professor of Biostatistics at the School of Medicine, Tulane University. To demonstrate significance, the original eleven pH values were consolidated into three groups (See Table 1).

The standard procedure developed, as described below, proved highly satisfactory for the hatching of miracidia of *S. japonicum*. A positive stool was secured as soon as possible after defecation and thoroughly comminuted in and, during the subsequent twelve-hour period, sedimented in water with a pH slightly below 6.6. The washings were continued until the supernatant water was completely clear and there was no odor of skatol in the sediment. When the fecal material did not settle readily, as was frequently the case with stools from the pig, Triton NE was added up to a concentration of 1 : 10,000 in the water used. Faust, Ingalls and See (1946) have pointed out that this will increase the rate of sedimentation; and although it swells the bulk of the residue, it does not appear to have any deleterious effect on the final miracidial yield. The clean supernatant water was decanted and the sediment poured into an Erlenmeyer flask fitted with a concavely carved rubber stopper holding an erect glass collecting tube. Alkaline water with a pH 7.6 or slightly above was added, almost to the top of the collecting tube. Flasks placed in a comparatively cool spot (25° C. to 30° C.) produced heavy yields of miracidia in from one to eight hours. When the water became foul or acid the production of miracidia declined and then ceased. By re-sedimenting the material in the flask for three decantations in water with an acid pH and then transferring it to another hatching flask containing alkaline water, repeated yields of miracidia were obtained.

SUMMARY

The results from studies of environmental conditions which might be expected to influence the hatching of *S. japonicum* eggs indicated there is no effect on the rate of hatching as a result of the degree of intensity of illumination, agitation of fecal matter in the suspension, or osmotic stimuli. No diurnal or nocturnal cyclic frequency in hatching rates was observed. A temperature range of 25° C. to 30° C. was found satisfactory. The work indicated that to obtain consistently good results it is most important that the fecal material be thoroughly washed and the water in the hatching flasks be clean and alkaline. The best miracidial yields were secured in water with pH 7.6. After hatching ceased in a flask, it was possible to obtain second and third yields by re-sedimenting the fecal material.

REFERENCES

BAUMAN, PRESTON M., BENNETT, H. J. AND INGALLS, J. W. 1948 The Molluscan Intermediate Host and Schistosomiasis Japonica. II. Observations on the Production and Rate of Emergence of Cercariae of *Schistosoma japonicum* from the Molluscan Intermediate Host, *Oncomelania quadrasi*. Amer. J. Trop. Med., (in press).

FAUST, ERNEST C., INGALLS, J. W. AND SEE, J. K. 1946 The Diagnosis of Schistosomiasis Japonica. III. Technics for the Recovery of the Eggs of *Schistosoma japonicum*. Amer. J. Trop. Med., **26**: 559–584.

FAUST, ERNEST C. AND MELENEY, H. E. 1924 Studies on Schistosomiasis Japonica. Amer. J. Hyg. Monographic Series No. 3, 339 pp.

MAGATH, THOMAS B. AND MATHIESON, D. R. 1946 Factors Affecting the Hatching of Ova of *Schistosoma japonicum*. J. Parasit., **32**: 64–68.

McMULLEN, DONALD B. AND BEAVER, P. C. 1945 Studies on Schistosome Dermatitis. IX. The Life Cycle of Three Dermatitis-producing Schistosomes from Birds and a Discussion of the Subfamily Bilharziellinae (Trematoda: Schistosomatidae). Amer. J. Hyg., **42**: 128–154.

MIYAGAWA, Y. 1916 Über die Veränderungen der Eier des *Schistosomum japonicum* unter den Einwirkungen verschiedener physikalischen und chemischen Agenzien und über einige prophylaktische Massregeln der Schistosomiasis. Mitt. med. Fak. Kaiserl. Univ. Tokyo, **15**: 453–475.

STUNKARD, HORACE, W. 1946 Possible Snail Hosts of Human Schistosomes in the United States. J. Parasit. **32**: 539–552.

SPONTANEOUS TOXOPLASMOSIS IN THE GUINEA-PIG IN PANAMA

Enid de Rodaniche and Teresina de Pinzon

Department of Public Health, Panama, R. P., and Gorgas Memorial Laboratory,
Panama, R. P.

Although toxoplasmosis has been identified in man and a wide variety of birds and mammals in various parts of the world, it was not until 1943 that its occurrence was first noted on the Isthmus of Panama. In that year Johnson described an epidemic of toxoplasmosis in Army carrier pigeons in the Canal Zone. These birds, however, represented imported stock and may have been infected at the time of introduction. A chronic form of this infection in pigeons has been demonstrated by Manwell and coworkers (1945). Recently, Kean and Grocott (1948) studied a case of congenital toxoplasmosis in a Panamanian infant and referred to 5 cases of asymptomatic infection with this organism observed by them in the Panama Area. These same authors (1945) in an earlier report of a case of probable sarcosporidiosis in a 48 year old Jamaican-born woman noted parasites in 5 of 60 laboratory guinea-pigs but were uncertain whether to classify them as sarcosporidia or toxoplasma. However, these animals also were recent importations from the States, where spontaneous toxoplasmosis in stock experimental guinea-pigs has been demonstrated by Sabin and Olitsky (1937) and Markham (1937).

During the course of studies of Q fever in the Gorgas Memorial Laboratory we observed spontaneous toxoplasmosis in guinea-pigs established in this area and without contact with recently imported members of their species. We feel that this finding is of especial interest from the epidemiological standpoint as guinea-pigs are frequently maintained as pets or as sources of food in homes both in the city and in rural areas where they come into intimate contact with human beings and domestic animals.

Due to the failure of other sources the Gorgas Memorial Laboratory recently was obliged to purchase guinea-pigs from native dealers who collect them from small breeders in the environs of the City of Panama and in the Interior of the Republic. One batch of 8 pigs purchased in the neighborhood of Panama Vieja on the outskirts of the city was noted to be of exceptionally poor quality, thin and sluggish with yellowish staring coat. One of these animals was found moribund the day after arrival. Post-mortem examination failed to reveal marked gross pathology but numerous toxoplasma were observed in Giemsa-stained impression smears of the liver, spleen, lung and peritoneal wall. Culture of the heart blood yielded colonies of gram-positive green-producing diplococci.

An emulsion of the spleen and part of the liver of this animal was used to inoculate intraperitoneally 2 mice which died in 24 hours. One was discarded due to advanced decomposition. Smears of the various organs of the other including spleen, liver, lung, brain, kidney, testicle and heart revealed large numbers of free and intracellular toxoplasma as well as overwhelming septicemia by gram-positive diplococci. An attempt to passage this strain to other guinea-pigs and mice was made by the simultaneous administration of sulfathiazole to control the bacterial in-

fection. However, we failed to find toxoplasma in animals sacrificed 1 to 3 weeks after injection. In this connection we may note that Sabin and Warren (1941) and Weinman and Berne (1944) have noted a marked prophylactic effect of the sulfonamide compounds against experimental toxoplasma infections in mice.

The remaining 7 guinea-pigs in the afore-mentioned batch improved in general condition and were later pressed into use for experimental work with *Rickettsia burneti*. Histological examination of these animals at the time of death or sacrifice revealed very light infection with toxoplasma in two. Attempts at further passage by the intraperitoneal route in adult and 10 day old guinea-pigs and adult white mice resulted in reproduction of the typical clinical syndrome of Q fever but in diminution in numbers or disappearance of the toxoplasma. Especially careful check was made of stained smears and serial sections of the various organs of these animals, as Mooser in 1929 and more recently Perrin and coworkers (1943) found that infections with toxoplasma complicated their studies of typhus in the guinea-pig. Subsequent purchases of guinea-pigs from other localities have revealed only one further toxoplasmic infection up to the present time.

Morphologically the toxoplasma observed by us correspond closely to the excellent descriptions of Wolf, Cowen and Paige (1940) and Guimarães (1943).

In Giemsa-stained contact smears these protozoons are readily identified (See Plate I). They are usually crescentic in shape with pointed or rounded ends, from 4 to 8 μ in length by 1.5 to 3 μ in width. The cytoplasm stains blue and the nucleus which is usually subterminal or terminal in position, round or band-shaped, stains a reddish purple. They may be observed free in the tissue fluid, in the act of penetrating the large mononuclear cells or in the intracellular position. Several may be observed penetrating the same cell simultaneously. In smears prepared several hours after the death of the animal the majority of toxoplasma were seen as shortened, thickened, oval, sharply defined forms with a vacuolated irregularly staining blue cytoplasm and no definite nucleus. These were considered to be degenerative forms. In paraffin-sections stained with hematoxylin-eosin, free forms were more difficult to identify but were occasionally noted in the sinuses of the liver and spleen. Intracellular clusters of the parasites or cysts, however, containing varying numbers of small organisms were readily observed. Where the number of intracellular organisms was few, a layer of cytoplasm usually surrounded each nucleus. But in cysts containing numerous organisms, the nuclei apparently were embedded in a syncytium. Toxoplasma were found in smears or sections of the liver, spleen, lungs, testicles, peritoneal wall, brain, kidney and heart. They were most abundantly present, however, in the liver, lungs and peritoneal wall.

PATHOGENICITY

We were unable to obtain any evidence of pathogenicity for this strain of toxoplasma by the intraperitoneal route of inoculation in either guinea-pigs or mice. Where symptoms or gross pathology was observed, there was always an associated infection with the diplococcus previously mentioned or with *R. burneti*. The animals employed may have possessed a certain degree of immunity. However, 10 day old guinea-pigs proved equally as resistant as older pigs. Infant mice were not available. The great abundance of toxoplasma noted in smears of animals dying of the diplococcal infection is of interest. Evidently this infection so reduced the natural re-

sistance of the animals as to permit rapid multiplication and widespread invasion by these protozoons.

CONCLUSION

Spontaneous infection with toxoplasma was observed in guinea-pigs purchased in the suburbs of the city of Panama, and representing stock established in this area and without contact with recently imported members of their species.

REFERENCES

GUIMARÃES, F. N. 1943 Toxoplasmose humana. Meningoencefalomielite toxoplasmica. Memorias Instituto Oswaldo Cruz **38**: 257–320.

JOHNSON, C. M. 1944 Annual Report of the Gorgas Memorial Laboratory, 1943 pp. 15–16. Government Printing Office, Wash., D. C.

KEAN, B. H. AND GROCOTT, R. G. 1945 Sarcosporidiosis or Toxoplasmosis in Man and Guinea Pig. Am. J. Path. **21**: 467–483.

————1948 Congenital Toxoplasmosis. J. Amer. Med. Ass. **136**: 104–108.

MANWELL, R. D., COULSTON, F., BINCKLEY, E. C. AND JONES, VIRGINIA. 1945 Mammalian and Avian Toxoplasmosis. J. Infec. Dis. **76**: 1–14.

MARKHAM, F. S. 1937 Spontaneous Toxoplasma Encephalitis in the Guinea Pig. Amer. J. Hyg. **26**: 193–196.

MOOSER, H. 1929 Tabardillo, an American variety of Typhus. J. Infec. Dis. **44**: 186–193.

PERRIN, T. L., BRIGHAM, G. D. AND PECKENS, E. C. 1943 Toxoplasmosis in Wild Rats. J. Infec. Dis. **72**: 91–96.

SABIN, A. B. AND OLITSKY, P. K. 1937 Toxoplasma and Obligate Intracellular Parasitism. Science **85**: 336–338.

———— AND WARREN, JOEL. 1941 Therapeutic Effect of the Sulfonamides on Infection by an Intracellular Protozoon (Toxoplasma). J. Bacteriol. **41**: 80.

WEINMAN, D. AND BERNE, R. 1944 Therapeutic Cure of Acute Experimental Toxoplasmosis in Animals. J. Amer. Med. Ass. **124**: 6–8.

WOLF, A., COWEN, D. AND PAIGE, BERYL. 1940 Toxoplasmic Encephalomyelitis. IV. Experimental Transmission of the Infection to Animals from a Human Infant. J. Exper. Med. **71**: 187–214.

Fig. 1.
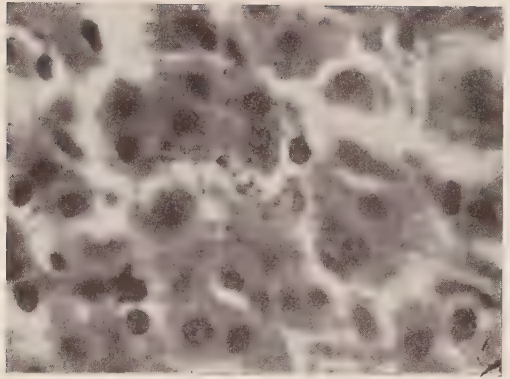
Fig. 2.

Fig. 3.

Fig. 4.
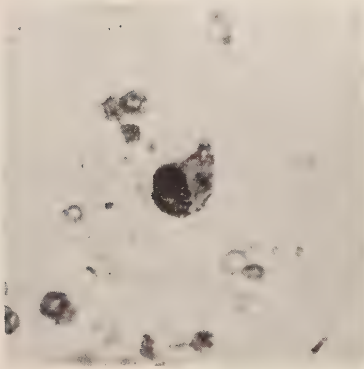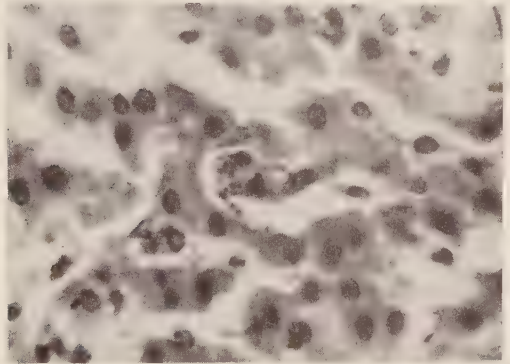
Fig. 5.
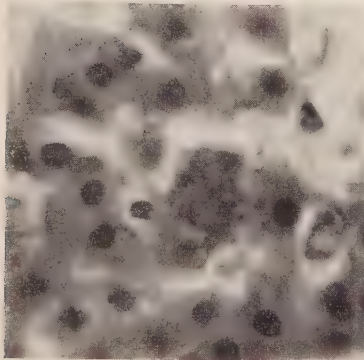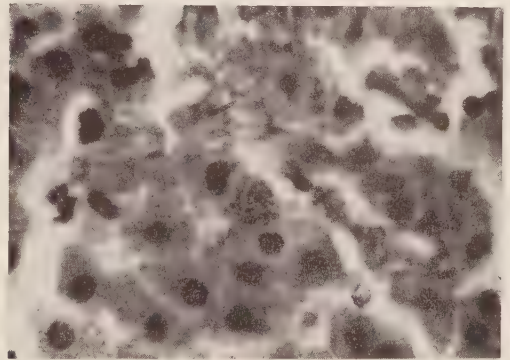
Fig. 6.

PLATE I

Contact smear of testicle of guinea-pig.   Giemsa.   ×720.

FIG. 1. Extracellular toxoplasma.
FIG. 2. Cluster of three intracellular toxoplasma.   One free parasite.

Paraffin section of liver of guinea-pig.   Hematoxylin and Eosin.   ×720.

FIG. 3. Large cyst.
FIG. 4. Toxoplasma free in sinus and invading contiguous cells.
FIG. 5. Small cluster of intracellular parasites.
FIG. 6. Small cyst.

155

A COMPARISON OF COMMON LABORATORY ANIMALS AS EXPERIMENTAL HOSTS FOR *SCHISTOSOMA MANSONI*[1]

Donald V. Moore, Tamarath K. Yolles, and Henry E. Meleney[2]

Department of Preventive Medicine, College of Medicine, New York University

In studying the various aspects of experimental schistosomiasis mansoni it was necessary to determine which of the common laboratory animals would produce the most suitable infection for studying its general course. In studying the action of chemotherapeutic agents against infections with *S. mansoni* it is desirable to know how many worms may be recovered from an animal after exposure to a known number of cercariae. Leiper (1918) found that a number of animals were susceptible to infection with *S. mansoni;* among these were white rats, Egyptian desert rats, black mice, guinea pigs, monkeys, and others not commonly used as laboratory animals. The primary purpose of Leiper's experiments was to differentiate between *S. mansoni* and *S. haematobium.* Many of his animals died from massive infections. On the other hand, in an attempt to produce light infections so as to insure the survival of the animals, many of the animals failed to develop any adult worms.

Since that time many investigators have used the common laboratory animals, including rabbits, as experimental hosts for various purposes. Most experimental animals have been infected by penetration of the cercariae through the skin. Cram and Bozicevich (1944) reported infections in rabbits, hamsters, guinea pigs, mice and monkeys with *S. mansoni* by the intraperitoneal injection of cercariae. The first report in which definite numbers of cercariae were used for infections is that of Cram and Files (1947) on the experimental infection of *S. mansoni,* by the intreperitoneal and percutaneous methods of exposure. They found that the intraperitoneal method gave poor results in rats, rabbits, and guinea pigs, but gave consistently good results in mice and hamsters.

Cram and Figgat (1947) carried out more detailed studies on the comparison of the two methods of infection in hamsters using *S. mansoni* and *S. japonicum.* Schubert (1948) used definite numbers of cercariae in chemotherapeutic experiments.

The purpose of this study was to determine the suitability of the common laboratory animals as experimental hosts for *S. mansoni.* The criteria used as a basis for comparing one onimal with another were: the percentage worm recovery following the administration of a known number of cercariae by intraperitoneal injection and by percutaneous exposure; the length of time required for the worms to reach maturity; the distribution of the adult worms in the host; and whether or not the infected animal passed viable eggs in the feces.

MATERIALS AND METHODS

Colonies of uninfected and infected *Australorbis glabratus* were established and maintained in the laboratory. Snails infected in the laboratory were usually exposed individually to from five to ten miracidia each.

In order to insure infections with both sexes of the worm, the cercariae from all lots of shedding snails were pooled and cercarial counts were made on the resulting cercarial suspension. The technique of counting varied only with the type of infection. For percutaneous infections, a 1 ml. pipette equipped with a rubber bulb was used for the removal of the cercariae from the suspension, and for the introduction of the known number of cercariae into the water to which the animals would be exposed. Prior to the removal of cercariae for counting or infecting, the water was agitated to insure an even distribution of cercariae. When water containing cercariae was allowed to stand quiescent for a short time, the cercariae concentrated near the surface. For counting, 0.5 ml. of cercarial suspension was removed and placed, drop by drop, on a glass plate $3\frac{1}{4}$ by $4\frac{1}{4}$ inches. With the aid of a dissecting microscope, the number of cercariae in each drop was determined, thus giving the number of cercariae in 0.5 ml. Generally, three such counts were made, and the average of these counts was used in calculating the number of cercariae per ml. of suspension.

When the infection was given by intraperitoneal injection, the counting technique was varied to determine the number of cercariae per ml. as delivered by the syringe and needle. For this purpose, a 5 ml. syringe equipped with a 22-gauge needle was used. Prior to counting the cercariae, the number of drops per ml. of water as delivered by the syringe-needle combination was determined. The method of computing the number of cercariae per ml. was the same as that described above. In discharging the cercarial suspension from the syringe, it was necessary to retain an air bubble in the syringe and to cause the air bubble to move back and forth, while the cercariae were being discharged, so that they would be evenly distributed throughout the liquid. By this method several consecutive animal injections could be given without refilling the syringe. This is essentially the same technique as that described by Schubert (1948) except that he used a 26-gauge needle, which we have found may decrease the number of cercariae actually delivered.

For the percutaneous infection of the smaller animals, such as mice, hamsters, rats, and guinea pigs, a glass jar of suitable size was prepared by placing in it an amount of spring water sufficient to immerse the posterior third of the animal and adding the necessary amount of cercarial suspension. The animal was placed in this jar and allowed to remain for thirty minutes. The water was then examined to see if any cercariae remained. If so, the animal was returned to the water for an additional thirty minutes. Each animal thus infected was allowed to dry individually under a lamp before being returned to the cage. Spring water was used instead of tap water in order to avoid the possible damaging effect of chlorine on the cercariae.

The above method is impractical for the percutaneous infection of rabbits, because of their large size. Therefore, a special technique was developed for these animals. The rabbit was tied supine to an animal board, and the ears pulled through a slot in the board. The necessary amount of cercarial suspension was added to spring water in a suitable glass container such as a half-pint fruit jar. The rabbit's ears were then immersed in the cercaria-infested water, and allowed to remain for

about thirty minutes.   If, at the end of this time, some of the cercariae remained in the water, the rabbit's ears were exposed for an additional thirty minutes.

The recovery of the schistosomes from infected animals was accomplished by the perfusion technique (Yolles *et al* 1947).

<div align="center">RESULTS</div>

MICE.   During the preliminary experiments, it was determined that 100 cercariae per mouse produced an infection which could be well tolerated and which at the same time produced quite extensive pathology.   In subsequent experiments, we have found that an infection level of 150 cercariae per mouse is also tolerated well, and will produce more worms per mouse.   This is a distinct advantage in experiments in which the recovery of the largest possible number of adult worms is desired.   As a result of extensive experimental infections in mice, we have found that infections with 100 to 150 cercariae per mouse produce the best results, and all of the mice become infected. Although, in these experiments, no attempt was made to determine the maximum worm load which mice can tolerate, Schubert (1948) found that the mortality in mice exposed to 250 cercariae was quite high.

Preliminary experiments on the suitability of albino mice as experimental hosts for *S. mansoni* suggested that the percentage worm recovery following intraperitoneal injection of the cercariae was considerably lower than the percentage recovery following percutaneous infection.   Subsequent carefully controlled experiments on mice infected with 100 cercariae per mouse revealed no significant difference in worm recovery between intraperitoneal inoculation and percutaneous infection (Table 1, Figure 1).   A group of 100 mice was used, fifty were infected by intraperitoneal inoculation of 100 cercariae each, and fifty by individual cutaneous exposure to 100 cercariae each.   Following intraperitoneal injection, an average of 21.4 per cent. of the cercariae injected were recovered as worms, and, following cutaneous exposure, 22.9 per cent. were recovered.   Schubert (1948) reports a 9 per cent. worm yield after intraperitoneal infection of mice using 100–150 cercariae.   In his series worm recovery from the mesenteric veins was accomplished by manually picking visible worms from these veins with fine forceps.   It is felt that this difference in worm yield would not be as great if he had used a technique of perfusion of these vessels under pressure.

Table 1 shows the progress of the development of the worms after intraperitoneal and percutaneous infections as well as the distribution of the worms in the mice. According to these data, the course of the infection is essentially identical following these two methods of infection.   Six weeks after infection 77 per cent. of the worms had reached maturity in the intraperitoneal group and 88 per cent. in those of the percutaneous group.   Eight weeks after infection 96 per cent. of the worms in the "intraperitoneal mice" and 99 per cent. in the "percutaneous mice" had reached maturity.   Ten weeks after infection, 100 per cent. of the worms in each group were mature.

From Table 1 it will be seen that four weeks after infection, the majority of the worms were found in the liver, 70 per cent. in the "intraperitoneal mice" and 94 per cent. in the "percutaneous mice".   From 6 to 12 weeks after infection, there is an approximately equal distribution of the worms between the liver and the mesenteric veins.   As the infection progresses, there is a tendency for more of the worms to be

MICE

TABLE 1.—*Recovery, maturity and distribution of S. mansoni following intraperitoneal and percutaneous infections*

Mode of Infection	Duration of Infection in Weeks	Number Cercariae per Mouse	Total Cercariae Used	No. of Mice	Number of Worms Recovered						Maturity of Worms Percentage		Distribution of Worms Percentage	
					Total	Mature		Immature			Mature	Immature	Liver	Mesentery
						Liver	Mes.	Liver	Mes.					
Intraperitoneal	4	100	1,000	10	173	16	19	105	33		20	80	70	30
	6	100	1,000	10	213	90	75	22	26		77	23	53	47
	8	100	1,000	10	211	115	87	3	6		96	4	56	44
	10	100	1,000	10	231	130	101	0	0		100	0	56	44
	12	100	1,000	10	242	96	146	0	0		100	0	40	60
	Total....		5,000	50	1,070									
	Percent Worm Recovery				21.4%									
Percutaneous	4	100	1,000	10	270	2	0	252	16		1	99	94	6
	6	100	1,000	10	190	65	104	19	2		88	12	44	56
	8	100	1,000	10	205	116	86	3	0		99	1	58	42
	10	100	900	9	247	104	143	0	0		100	0	42	58
	12	100	1,000	10	209	78	131	0	0		100	0	37	63
	Total....		4,900	49	1,121									
	Percent Worm Recovery				22.9%									

found in the mesenteric veins. Twelve weeks after infection, approximately 60 per cent. of the worms were recovered from the mesenteric veins.

Fecal examinations for eggs were made on groups of infected mice and on individual mice by using the following techniques: direct smear, hatching, and Stoll count. The examinations were started 4 weeks after infection, and continued at half-week intervals up to 17 weeks, after which time they were made only once a week. Regardless of the mode of infection, viable eggs appeared in the feces of infected mice between 6 and 6½ weeks after infection. After egg passage began, it con-
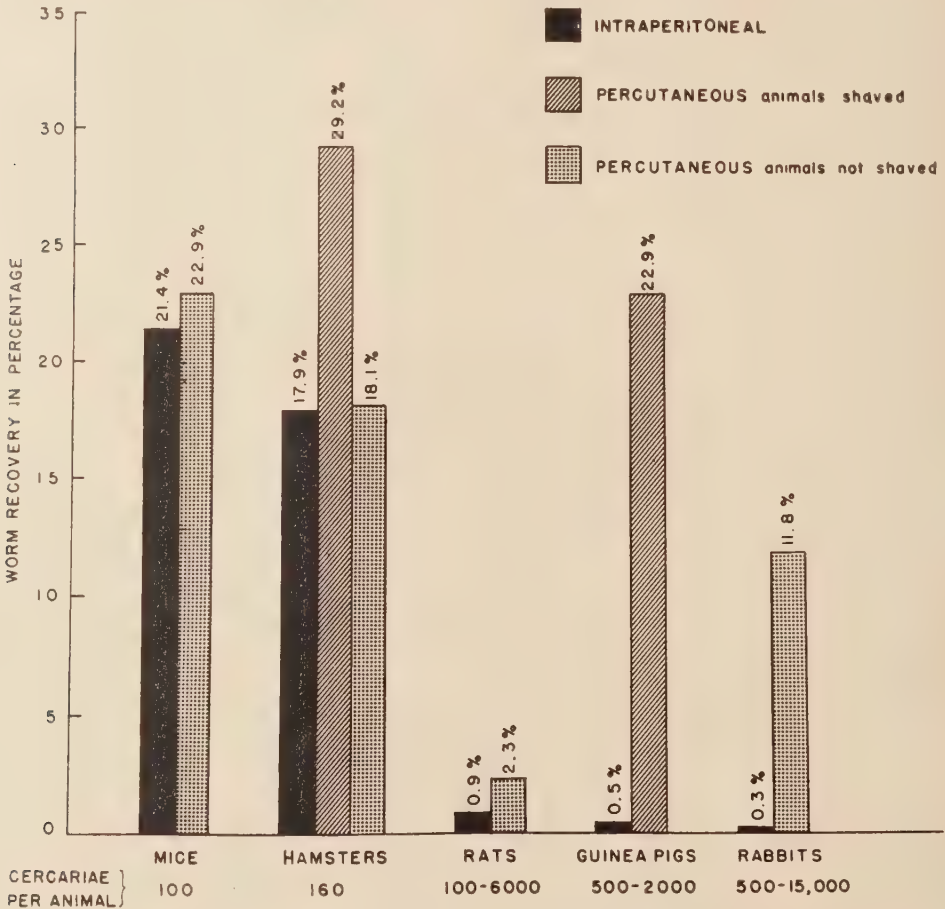
Fig. 1. Comparison of worm yield following intraperitoneal and percutaneous infection of animals with *S. mansoni*.

tinued without interruption until the death of the mouse. One mouse regularly passed viable eggs in the feces until it died one year and two weeks after infection.

One group of research workers studying the chemotherapy of schistosomiasis were using a mixed strain of gray and black mice instead of albino mice for experimental animals. They were under the impression that egg passage was transitory in the mice they were using. We obtained a group of their mice and infected them in this laboratory to compare the egg passage and the worm recovery with similar observations in the albino mice which we were using routinely. Egg counts of the

pooled feces of each group of mice were made at half-week intervals from 7 to 17 weeks after infection, and thereafter at weekly intervals until the death of the mice. Table 2 gives the results of the averages of the egg counts made on these two groups of mice. Egg passage began at $6\frac{1}{2}$ weeks after infection in both groups and continued until the death of the mice. The last mouse in the albino group died 38 weeks after infection and the last mouse in the mixed strain of gray-black mice died 18 weeks after infection. The average egg count per mouse in single examinations varied from 100 to 1200 per ml. of feces.

The gross pathology produced by the infection in mice varies with the age of the infection, the egg tubercles in the liver and in the intestinal wall becoming more pronounced as the age of the infection increases. Four weeks after exposure the gross pathology is either slight or non-existent. The liver is either normal in appearance, or shows a few whitish spots just beneath the surface. At this stage of infection the worms are not usually visible through the wall of the portal vein. The walls of the intestinal tract do not exhibit any gross pathology. The spleen is either normal or slightly enlarged. The lungs are normal in appearance.

TABLE 2.—*Average egg counts from albino mice and gray mice, each infected percutaneously with 100 cercariae of* S. mansoni

Duration of Infection in Weeks	Albino Mice Average Eggs/ml Feces/Mouse	Gray Mice Average Eggs/ml Feces/Mouse
7 – 8	455	389
$8\frac{1}{2}$–10	741	537
$10\frac{1}{2}$–12	833	616
$12\frac{1}{2}$–16	620	637
17 –20	365	115
21 –24	565	*
25 –28	441	
29 –32	516	
33 –36	633	
37 –38	666	

* Last mouse died in eighteenth week.

Six weeks after infection, the liver shows from few to many grayish white spots, usually slightly depressed, giving the surface of the liver a roughened appearance. Worms are usually visible through the walls of the portal and mesenteric veins. The walls of the intestine are usually normal in appearance, but in some mice minute typical egg tubercles may be observed. Eggs may be found in the liver and in some areas of the intestinal wall by microscopic examination of tissue press preparations. The size of the spleen varies from slightly larger than normal to twice normal. The lungs are usually still normal in appearance.

Eight weeks after infection, the liver has a gray, mottled appearance with frequent or numerous white spots. The worms are plainly visible in the portal and mesenteric veins. Egg tubercles in the wall of the intestine may or may not be visible. Eggs can be found microscopically in the liver and in some areas of the intestinal wall. The spleen is enlarged, its size ranging from $1\frac{1}{2}$ to 3 times normal. The lungs of some mice are normal in appearance and others show lesions due to schistosome eggs. In 15 out of 20 mice autopsied at eight weeks clear brownish raised spots, pinhead in size, were observed on the surface of the lungs. The number of spots ranged from 1 to 4 or 5. Microscopic examination of these spots revealed the presence of a single egg in each spot.

Ten weeks after infection, the liver shows some enlargement and the white spots

indicative of egg tubercles are numerous. Worms are visible in the portal and mesenteric veins. Egg tubercles are visible throughout the length of the intestinal wall in all mice, more numerous in some than in others. Eggs can be seen microscopically in the liver of all mice and in all parts of the intestinal tract. The spleen is enlarged and ranges from $2\frac{1}{2}$ to 3 times normal size. The lungs were normal in 7 out of 19 mice autopsied, the remaining 12 mice had from 1 to 10 brownish clear spots on the surface of the lungs.

Twelve weeks after infection the liver is definitely enlarged in some of the mice, the whitish spots are coalesced to form larger grayish areas giving the liver a roughened, grayish appearance. Worms are easily observed in the portal and mesenteric veins. Egg tubercles in the wall of the intestine are usually sufficiently abundant so as to give the outer surface a roughened appearance. The spleen ranges in size from $2\frac{1}{2}$ to $3\frac{1}{2}$ times normal. In several of the mice, 2 or 3 discrete white spots were observed on the surface of the spleen. Microscopic examination revealed the presence of eggs in the spleen tissue. The lungs were normal in only one mouse in twenty autopsied at this age of infection. The remainder all had clear brownish spots on the lungs ranging from 5 or 6 to 15 or more.

The frequency and the number of lesions due to eggs in the lungs seems to increase with the age of the infection. In a few instances the presence of eggs in the lungs was accompanied by the presence of adult worms, however, since eggs have been found in the lungs on many occasions when no worms could be recovered from the lungs, eggs must be carried to the lungs by the blood stream. This could be accomplished either by a penetration of the capillary barrier in the liver or through a portal-systemic anastomosis such as the hemorrhoidal. In a few mice we have found immature or adult worms in the blood vessels of the lungs. The presence of these worms in the lungs signifies either that the young worms were trapped in the lungs during some phase of their migration and continue to develop there or that an occasional adult worm migrates from the portal to the systemic circulation and is carried in that circulation to the lungs. Worms recovered from the lungs are usually folded back on themselves and are somewhat smaller than adult worms normally found in the hepatic or mesenteric circulation. In some cases, worms in the lungs may be detected grossly by the appearance of small black streaks in the lung tissue representing the hematin in the gut of the worm.

HAMSTERS. Preliminary comparisons of worm recovery in hamsters (*Cricetus auratus*), following intraperitoneal and percutaneous infections, indicated that worm recovery after intraperitoneal infection was higher than after percutaneous exposure. Since this was contrary to the results obtained in mice, it was thought that perhaps the relatively thicker and longer fur of the hamster interfered with the penetration of the cercariae. Therefore a second series consisting of 70 hamsters was infected. This series was divided into three groups: (1) infection by intraperitoneal route; (2) percutaneous exposure following the removal of the fur from the posterior third of the hamster by clipping; (3) percutaneous exposure without removal of the fur. The results obtained from this second series indicated that the fur of the hamster did interfere with the penetration of the cercariae (Table 3, Figure 1). The percentage worm recovery following these three methods of infection was as follows: intraperitoneal injection 17.9 per cent.; shaved skin percutaneous infection 29.2 per cent.; unshaved skin percutaneous infection 18.1 per cent. Thus in hamsters as in

HAMSTERS

TABLE 3.—*Recovery, maturity and distribution of S. mansoni following intraperitoneal, shaved percutaneous and unshaved percutaneous infections*

Mode of Infection	Duration of Infection in Weeks	Number Cercariae per Hamster	Number of Hamsters	Total Cercariae Used	Number of Worms Recovered						Maturity of Worms Percentage		Distribution of Worms Percentage	
					Total	Mature		Immature			Mature	Immature	Liver	Mesentery
						Liver	Mes.	Liver	Mes.					
Intraperitoneal	4	160	4	640	135	28	18	70	19		34	66	73	27
	6	160	8	1,280	261	170	61	22	8		88	12	74	26
	8	160	5	800	126	83	29	12	2		89	11	75	25
	10	160	4	640	90	59	29	2	0		98	2	68	32
	12	160	2	320	47	17	23	1	6		85	15	38	62
	Total....		23	3,680	659									
	Percent Worm Recovery				17.9%									
Shaved Percutaneous	4	160	3	480	120	11	4	88	17		12	88	82	18
	6	160	7	1,120	412	233	111	56	12		84	16	70	30
	8	160	5	800	232	165	46	13	8		91	9	77	23
	10	160	4	640	170	139	30	0	0		99	1	82	18
	12	160	3	480	95	57	27	11	0		88	12	72	28
	Total....		22	3,520	1,029									
	Percent Worm Recovery				29.2%									
Unshaved Percutaneous	4	160	4	640	125	20	7	82	16		22	78	82	18
	6	160	7	1,120	200	125	59	12	4		92	8	68	32
	8	160	6	960	137	102	31	0	4		97	3	74	26
	10	160	5	800	199	153	39	5	2		96	4	79	21
	12	160	3	480	63	39	20	3	1		94	6	67	33
	Total....		25	4,000	724									
	Percent Worm Recovery				18.1%									

mice there is no significant difference in the percentage worm recovery following intraperitoneal and unshaved percutaneous infections. When the fur is removed from the portion of the hamster exposed to cercariae a higher percentage of adult worms is recovered. This difference is statistically significant. Our results compare favorably with those of Cram and Figgat (1947) with regard to the comparative recovery of *S. mansoni* in hamsters. They obtained 19 per cent. recovery following intraperitoneal injection, and 33 per cent. following shaved skin percutaneous infection.

In our experiments the hamsters were exposed to 160 cercariae each, and adult worms were recovered from all animals. This level of infection produces definite gross pathology but it is not as extensive as in mice infected with a similar number of cercariae. Subsequent experimental infections in this laboratory have shown that hamsters will tolerate 300 to 350 cercariae per animal. We did not attempt to determine the maximum cercarial dose tolerated by hamsters. Cram and Files (1947) noted that with larger doses of cercariae a number of hamsters died during the prepatent stage of the infection.

The mode of infection seems to have no apparent effect on the length of time required for the infection to mature in hamsters (Table 3). The rate at which the worms mature in hamsters compares favorably with the rate of maturation in mice (Table 1). It will be noted in Table 3 that 12 weeks after infection the percentage of mature worms is lower in each case than that observed in the animals autopsied 10 weeks after infection. In the animals autopsied 12 weeks after infection we found that there were more female worms than male worms, and that all of the immature worms were females. This will be discussed more fully in a future publication dealing with the sexual development of *S. mansoni*.

With regard to the distribution of the worms between the intra-hepatic and mesenteric veins, Table 3 shows that a larger percentage of the worms was recovered from the liver than from the mesenteric veins, with the exception of the twelfth week group of "intraperitoneal" animals. However the number of worms present in the mesenteric veins is sufficient to produce gross pathology in the intestinal wall.

Viable eggs may be found in the feces of infected hamsters 5 to 6 weeks after infection. Our experience leads us to believe that egg passage will continue without interruption as long as the infected hamster lives.

The general appearance of the gross pathology in hamsters is essentially identical with that described for mice. The spleen of infected hamsters usually does not show any increase in transverse diameter but does increase in length. There seems to be a greater tendency for eggs to lodge in the spleen of hamsters than in the spleen of mice. The lesions produced by eggs in the lung tissue are identical with those seen in mice, and the number of lesions and frequency of occurrence increase with the age of the infection. Occasional adult worms have been found in the lungs of hamsters.

RATS. (Table 4, Figure 1). In a group of 27 rats infected by intraperitoneal injection, the average worm recovery per rat was 0.9 per cent. and the highest in any one animal was 1.75 per cent. In a group of twelve rats infected by percutaneous route the average worm recovery was 2.3 per cent. and the highest in any one animal was 5 per cent. The rats used in this series were exposed to varying numbers of cercariae ranging from 100 to 6000 per rat. Regardless of the mode of exposure and number of cercariae, some rats yielded no worms.

The rate of development and the distribution of the worms in the rat is quite dif-

RATS

TABLE 4.—*Recovery, maturation, and distribution of S. mansoni following intraperitoneal and percutaneous infection*

Mode of Infection	Duration of Infection in Weeks	Number Cercariae per Rat	No. of Rats	Total Cercariae Used	Number of Worms Recovered					Maturity of Worms Percentage		Distribution of Worms Percentage	
					Total	Mature		Immature		Mature	Immature	Liver	Mesentery
						Liver	Mes.	Liver	Mes.				
Intraperitoneal	5–6	250	4	1,000	5	4	0	1	0	80	20	100	0
	7–8	100	4	400	5	0	0	5	0	0	100	100	0
		250	3	750	2	0	0	2	0				
		500	6	3,000	23	*	*	*	*	*	*	*	*
		1000	2	2,000	41	*	*	*	*	*	*	*	*
	10	250	3	750	3	2	0	1	0	67	33	100	0
	14	250	5	1,250	2	2	0	0	0	100	0	100	0
	Total		27	9,150	81								
	Percent Worm Recovery				0.9%								
Percutaneous	6	500	2	1,000	22	15	0	7	0	30	70	99	1
		6,000	2	12,000	307	84	0	220	3				
	7–8	250	1	250	8	1	0	7	0	12	88	100	0
		500	1	500	0	0	0	0	0				
	9–10	250	1	250	8	7	0	1	0	89	11	100	0
		500	1	500	1	1	0	0	0				
	12+	500	3	1,500	0	0	0	0	0	0	0	0	0
	Unknown	1,000	1	1,000	50	16	3	27	4	38	62	86	14
	Total		12	17,000	396								
	Percent Worm Recovery				2.3%								

* Maturity and Distribution of Worms not determined.

ferent than in mice and hamsters. There is a suggestion that the worms mature more slowly in the rat and that mature worms are considerably smaller than those from mice and hamsters. It will also be noted in Table 4 that in only two instances were any worms found in the mesenteric veins.

Repeated attempts have failed to reveal any eggs in the stools of infected rats or even eggs in the intestinal walls. These findings are in accordance with the findings of Koppisch (1937). The gross pathology in rats is essentially limited to the liver. It was never as extensive as that seen in mice and hamsters. Splenomegaly was not observed in the rats. These observations indicate that the rat is not a favorable host for *S. mansoni.*

GUINEA PIGS

TABLE 5.—*Recovery, maturation and distribution of* S. mansoni *following intraperitoneal and shaved percutaneous infection*

Mode of Infection	Duration of Infection in Weeks	Number Cercariae per Guinea Pig	No. Guinea Pigs	Total Cercariae Used	Number of Worms Recovered					Maturity of Worms Percentage		Distribution of Worms Percentage	
					Total	Mature		Immature		Mature	Immature	Liver	Mesentery
						Liver	Mes.	Liver	Mes.				
Intraperitoneal	6	1,000	1	1,000	13	0	0	13	0	0	100	100	0
	8	1,000	1	1,000	0	0	0	0	0	0	0	0	0
	10	1,000	1	1,000	0	0	0	0	0	0	0	0	0
	12	1,000	1	1,000	6	3	3	0	0	100	0	50	50
	Total		4	4,000	19								
	Percent Worm Recovery	...		.....	0.5%								
Shaved Percutaneous	4	500	1	500	26	0	0	24	2	0	100	92	8
	6	500	1	500	66	40	19	5	2	62	38	86	14
		1,000	1	1,000	218	139	33	46	0				
		2,000	1	2,000	106	11	0	95	0				
	8	500	1	500	60	40	13	7	0	82	18	88	12
		1,000	1	1,000	209	193	1	15	0				
		2,000	1	2,000	319	182	53	81	3				
	10	1,000	1	1,000	187	102	26	59	0	71	29	49	51
		2,000	1	2,000	545	200	190	0	155				
	12	1,000	1	1,000	157	82	67	8	0	95	5	57	43
		2,000	1	2,000	1,194*	182	423	0	0	100*	0	15*	36*
	Total		11	13,500	3,087								
	Percent Worm Recovery	...		.....	22.9%								

*Total includes 589 mature worms (49% of total) recovered from lungs.

GUINEA PIGS. (Table 5, Figure 1). Our data on guinea pigs are limited because a large proportion of these animals died of other causes during the course of the experiments. A group of 4 guinea pigs was given intraperitoneal injections of 1000 cercariae each; two of these animals yielded no worms at autopsy and two yielded 13 and 6 worms respectively, thus giving an average worm recovery of 0.5 per cent. A group of 11 guinea pigs was infected by the percutaneous method after clipping the hair from the posterior third of the animals. Cercarial dosages of 500, 1000, and 2000 cercariae per animal were used. The percentage worm recovery following percutaneous infection ranged from 5 to 60 per cent. with an average of 22.9 per cent.

As in rats, the maturation of the worms in guinea pigs was slow. Twelve weeks were required before most of the worms became mature. The distribution of the worms in the host is also indicative of the slow rate of maturation. Prior to ten weeks after infection, the majority of the worms were recovered from the liver. Ten and twelve weeks after infection, the distribution of the worms between the liver and mesenteric veins was nearly equal. As in rats, the mature worms were smaller than those recovered from mice and hamsters.

In spite of the fact that mature female worms were found in the mesenteric veins, and that eggs were demonstrated in the intestinal wall, eggs were never found in the .

RABBITS

TABLE 6.—*Recovery, maturation, and distribution of* S. mansoni *following intraperitoneal and percutaneous infection*

Mode of Infection	Duration of Infection in Weeks	Number Cercariae per Rabbit	No. of Rabbits	Total Cercariae Used	Number of Worms Recovered					Maturity of Worms Percentage		Distribution of Worms Percentage	
					Total	Mature		Immature					
						Liver	Mes.	Liver	Mes.	Mature	Immature	Liver	Mesentery
Intraperitoneal	6	500	2	1,000	21	12	1	7	1	75	25	75	25
		10,000	1	10,000	47	24	14	8	1				
	7–8	1,000	1	1,000	16	8	4	4	0				
		2,000	2	4,000	81	25	24	16	16				
		5,000	2	10,000	0	0	0	0	0	63	37	55	45
		10,000	2	20,000	0	0	0	0	0				
		15,000	1	15,000	0	0	0	0	0				
	9	3,700	1	3,700	14	11	0	3	0	79	21	100	0
	Total		12	64,700	179								
	Percent Worm Recovery			.....	0.3%								
Percutaneous	6	500	2	1,000	167	77	47	43	0				
		1,000	1	1,000	186	95	69	17	5	59	41	89	11
		15,000	2	30,000	2,032	1,077	52	806	97				
	7–8	1,000	1	1,000	307	132	126	41	8				
		2,000	2	4,000	706*	334	120	216	28				
		5,000	1	5,000	578	130	253	17	178	67	33*	53*	47*
		9,000	1	9,000	1,960	222	902	796	40				
		10,000	1	10,000	666	338	265	1	62				
	9	10,000	1	10,000	1,797	545	308	899	45	47	53	80	20
	Total		12	71,000	8,399								
	Percent Worm Recovery			.....	11.8%								

* Total includes 8 immature worms (0.2% of total) recovered from lungs of one rabbit.

feces of our guinea pigs. Fecal examinations, including the hatching technique, were made on all infected guinea pigs from 4½ weeks through 12 weeks after infection without finding any eggs.

The gross pathology observed in infected guinea pigs was essentially identical with that seen in mice and hamsters, except that it was never so extensive. The spleen did not show any enlargement nor were eggs found in it. Lung pathology attributable to schistosomiasis was not observed except in one guinea pig, which is worthy of separate consideration. This animal was exposed to 2000 cercariae by percutaneous infection, and was autopsied twelve weeks later. No eggs were found at any time although feces were examined twice a week beginning with the fourth

week of infection. At autopsy, the lungs showed many clear brownish raised areas about 1 mm in diameter. The general appearance of the lung tissue was grayish black. The liver was small and the surface was roughened and mottled by many grayish white spots. The spleen was normal in size, but very dark in color due to hematin. Numerous tubercles were observed on the wall of the intestine. Some of these tubercles were hemorrhagic. Many eggs were found in press preparations of the lungs, liver, stomach, duodenum, jejunum, ileum, colon and rectum. A total of 1,194 worms (60 per cent. of the cercariae employed) were recovered from this animal by perfusion with the following distribution: liver 182, mesenteric veins 423, lungs 589.

RABBITS. (Table 6, Figure 1). Twelve rabbits were given intraperitoneal injections of 500 to 15,000 cercariae per rabbit. The highest worm recovery was 4 per cent., the average 0.3 per cent. Of the 12 rabbits thus infected, 7 yielded no worms at autopsy. This failure of intraperitoneal infection in rabbits has been made the subject of a separate study (Yolles, Moore and Meleney, in preparation).

Twelve rabbits were given infections by immersing the ears in cercaria-infested water. The number of cercariae per rabbit ranged from 500 to 15,000. The worm recovery ranged from 3 to 30 per cent., with an average of 11.8 per cent. All of the rabbits infected in this manner harbored worms when autopsied.

As in rats and guinea pigs, the worms required more time to reach maturity in rabbits and the mature worms were much smaller than from mice and hamsters. The percentage of worms reaching maturity in rabbits within 6 to 9 weeks varied, but did not seem to be related to the mode of infection or to the number of worms present in any one rabbit. If the infection had been allowed to extend to twelve weeks, undoubtedly a greater percentage of the worms would have reached maturity. The worms were well distributed between the liver and the mesenteric veins. In all of the rabbits observed, more worms were found in the liver than in the mesenteric vessels, but considering the percentage of worms which were mature, their distribution is comparable to that in mice and hamsters.

Repeated attempts to demonstrate eggs in the feces of infected rabbits failed. This is in accordance with the findings of Koppisch (1937). He found eggs in the intestinal walls, but did not observe any ulceration due to eggs in any of his rabbits.

The gross pathology in infected rabbits was less marked than that seen in mice, hamsters and guinea pigs. The liver damage did not seem to be as extensive as in mice and hamsters. The egg tubercles in the liver were best observed along the margins of the lobes. Rabbits differed from mice, hamsters and guinea pigs in that egg tubercles were not observed grossly in the intestinal wall although tissue press examinations of the intestinal wall revealed numerous eggs. Eggs were never observed in the spleen tissue, although splenomegaly was observed. Eggs and both immature and mature worms were occasionally found in the lungs.

CONCLUSIONS

The ideal experimental host for *Schistosoma mansoni* is one in which the worms develop to maturity in a reasonable length of time, produce viable eggs which are excreted in the feces and produce pathological changes similar to those produced in man. In addition it is desirable to use an animal which is readily available and to know whether intraperitoneal injection or cutaneous exposure yields better results

as far as maturation of the worms and percentage worm recovery are concerned. In chemotherapeutic studies it is important to know the age at which the worms reach maturity, and the expected worm yield following a particular method of infection. The intraperitoneal injection of cercariae has some advantages over cutaneous exposure in that large numbers of animals may be infected at one time with a minimum of laboratory personnel, space and equipment.

The results of our experiments are in agreement with those of Cram and her co-workers in indicating that mice and hamsters are the most suitable of the common laboratory animals as experimental hosts for *S. mansoni*. The prepatent period in both of these animals is from 5 to 6 weeks. The majority of the worms are mature 8 weeks after infection, viable eggs are easily found in the feces, and the animals continue to pass eggs as long as they live. In one instance we observed continuous egg passage in a mouse for over a year. On the basis of percentage worm recovery, mice and hamsters are equally suitable as hosts for *S. mansoni*. There is no significant difference in the percentage worm recovery in mice following intraperitoneal and percutaneous infections. The same is true of hamsters infected percutaneously when the fur is not removed. However, when hamsters are shaved prior to cutaneous exposure the precentage worm recovery is significantly greater, and is also significantly greater than in mice after either intraperitoneal or percutaneous infection. The choice of mice or hamsters as experimental hosts for *S. mansoni* rests with the individual investigator.

Rats, guinea pigs, and rabbits are unsatisfactory as experimental hosts for *S. mansoni* for several reasons. In these animals the worms mature more slowly than they do in mice and hamsters, and when they do reach maturity the eggs produced are not passed in the feces. Therefore these animals cannot be used for the maintenance of a stock infection to provide material for the infection of snails. Neither do they have any value in appraising chemotherapeutic agents if the presence or absence of eggs in the feces is to be used as a criterion. In all of these animals few or no worms are obtained after intraperitoneal infection. Large numbers of worms may be obtained from guinea pigs and rabbits following percutaneous infection but the mature worms are considerably smaller than those obtained from mice and hamsters.

Guinea pigs and rabbits may serve a limited purpose in that large numbers of worms may be obtained from these animals for antigen or serological studies. These animals may be infected with thousands of cercariae and large numbers of mature worms obtained 12 or more weeks after infection. The animals tolerate such infections without apparent ill effects. The gross pathology is never as extensive as that produced in mice and hamsters infected with smaller numbers of worms.

Of all the animals used in this study the rat is the poorest host. In this animal the worms usually remain in the intra-hepatic circulation, and some rats seem to be refractory regardless of the method of infection.

SUMMARY

The common laboratory animals (mice, hamsters, rats, guinea pigs, and rabbits) were infected with *S. mansoni* by two methods: intraperitoneal injection and cutaneous exposure. The course of the resulting infection was studied and the following points used as a basis for comparing the suitability of the animals as hosts for *S. mansoni*: percentage worm recovery, time required for the worms to reach maturity, and the presence of viable eggs in the feces.

1. MICE. Animals infected with 100 cercariae yielded an average of 21.4 per cent. worm recovery following intraperitoneal infection and 22.9 per cent. following percutaneous infection. Eight weeks after infection 96–99 per cent. of the worms were mature. Viable eggs appeared in the feces 6–6½ weeks after infection and egg passage continued until the death of the animals.

2. HAMSTERS. Anmials infected with 160 cercariae yielded an average of 17.9 per cent. worm recovery after intraperitoneal infection, 18.1 per cent. after unshaved percutaneous infection, and 29.2 per cent. after shaved percutaneous infection. Eight weeks after infection 89–97 per cent. of the worms were mature. Viable eggs appeared in the stool 5–6 weeks after infection and egg passage continued until the death of the animals.

3. RATS. Animals infected with 100–6000 cercariae yielded an average of 0.9 per cent. worm recovery following intraperitoneal infection and 2.3 per cent. after percutaneous infection. Ten weeks after infection 67–89 per cent. of the worms were mature, but were smaller than in mice and hamsters. Eggs were never demonstrated in the feces at any time.

4. GUINEA PIGS. Animals infected with 500–2000 cercariae yielded an average of 0.5 per cent. worm recovery following intraperitoneal infection and 22.9 per cent. after shaved percutaneous infection. Eight weeks after infection 82 per cent. of the worms were mature, but were smaller than in mice and hamsters. Eggs were never demonstrated in the feces at any time.

5. RABBITS. Animals infected with 500–15,000 cercariae yielded an average of 0.3 per cent. worm recovery following intraperitoneal infection and 11.8 per cent. after percutaneous infection. Seven to 8 weeks after infection 63–67 per cent. of the worms were mature, but were smaller than in mice and hamsters. Eggs were never demonstrated in the feces at any time.

6. It is concluded that mice and hamsters are the most suitable common laboratory animals for experimental infections with *S. mansoni*. Rats are unsatisfactory hosts because very few worms develop to maturity. Guinea pigs and rabbits are of value only for producing large numbers of worms, and must be infected by percutaneous exposure.

REFERENCES

CRAM, E. B. AND BOZICEVICH, J. 1944 Experimental schistosomiasis mansoni infection by intraperitoneal injection. Trop. Med. News **1**: 16–17.

CRAM, E. B. AND FIGGAT, W. B. 1947 Experimental mammalian infection with the schistosomes of man. II. Comparative study of *Schistosoma mansoni* and *Schistosoma japonicum* infections produced by immersion and intraperitoneal injecton. Nat. Inst. Health Bull. 189. pp. 106–108.

CRAM, E. B. AND FILES, V. S. 1947 Experimental mammalian infection with the schistosomes of man. I. Laboratory animals as a source of supply of adult schistosomes and their ova. Nat. Inst. Health Bull. 189. pp. 101–105.

KOPPISCH, E. 1937 Studies on schistosomiasis mansoni in Puerto Rico. IV. The pathological anatomy of experimental schistosomiasis mansoni in the rabbit and albino rat. Puerto Rico J. Pub. Health and Trop. Med. **13**: 1–54.

LEIPER, R. T. 1918 Report on the results of the bilharzia mission in Egypt, 1915. J. Roy. Army Med. Corps. **30**: 235–260.

SCHUBERT, M. 1948 Conditions for drug testing in experimental schistosomiasis mansoni in mice. Am. J. Trop. Med. **28**: 121–136.

YOLLES, T. K., MOORE, D. V., DEGUISTI, D. L., RIPSOM, C. A., AND MELENEY, H. E. 1947 A technique for the perfusion of laboratory animals for the recovery of schistosomes. J. Parasit. **33**: 419–426.

A NEW FLEA FROM THE POCKET GOPHER

R. B. EADS AND G. C. MENZIES

Bureau of Laboratories, Texas State Department of Health, Austin, Texas

Three males of an apparently undescribed species of *Dactylopsylla* Jordan were taken while examining a series of skins of the chestnut-faced pocket gopher, *Cratogeomys castanops* (Baird). The gophers were collected during the summer of 1947 by Dr. Frank W. Blair, University of Texas Mammalogist, from Armstrong County, Texas. In June 1948, students of Dr. Blair obtained twenty-two additional specimens from the same species of gopher taken in Presidio County. Mr. Frank M. Prince kindly furnished a male and female flea from *Thomomys* with the same specific characteristics and paratypes of the related species, *Dactylopsylla neomexicana* Prince, for comparison.

Dactylopsylla percernis, n. sp.

Holotype Male

HEAD. Subantennal lobes of the genae acuminate; interantennal ridge not prominent; clypeal tubercle inconspicuous; labial palpi five segmented and not reaching apex of fore coxae; eyes absent but eye position marked by sclerified ring; preantennal region with an irregular lower row of seven large bristles (paratypes with five to seven), and an upper row of seven smaller bristles (paratypes with six to eight); postantennal region with an irregular row of about thirty fine setae and two bristles, a large and small one, along the margin of the antennal groove in addition to a row of seven bristles on the posterior margin.

THORAX. Pronotum with a row of bristles and a comb of twenty-four spines; metanotum with two regular rows of bristles on apical two-thirds and irregularly covered with smaller bristles on basal third; episternum of pleuron of mesothorax separated from epimeron by a prominent pleural ridge; metanotum with three rows of bristles and one pale apical spine; front coxae with numerous stout bristles; front femora with about twenty laterally placed slender bristles; mid and hind femora with twelve to fifteen lateral bristles in a row; tibia with very stout bristles, apical ones longer than the first segment of the tarsi; last tarsal segment with four pairs of stout bristles in a row and a basal pair out of line with the others and closer together.

MODIFIED SEGMENTS OF ABDOMEN. Two antepygidial bristles present, one much longer than the other; VIII sternite reduced in size, with four large bristles on ventral border toward apex; IX sternite with apical lobe trumpet-shaped, basal lobe not well defined and clothed with fine bristles; process of clasper short, broad, with four small bristles toward evenly rounded apex; movable finger of the clasper about twice as long as process of clasper, posterior border curving sharply toward acuminate apex and forming a structure resembling the head of a bird, a large and three small bristles present at apex of finger, four others on posterior border.

FEMALE. MODIFIED SEGMENTS. Three antepygidial bristles on each side, two long and one short; anal stylet over three times as long as wide at base and tapering toward apex, a long bristle on ventral margin halfway from base to apex and another two-thirds of the distance, a very long bristle at apex; VII sternite divided into two lobes by a moderate sinus, upper lobe poorly defined and merging into the oblique dorsocaudal margin (paratypes show considerable variation in the depth of the sinus and in the shape of the two lobes), the sternite with a regular row of heavy bristles and a less regular row of smaller ones, body of spermatheca not as wide as long, arm shorter than body and well sclerified, especially at apex.

Type host: *Cratogeomys castanops* (Baird)
Type locality: Presidio County, Texas

Holotype male and allotype female collected in Presidio County, Texas, June 1948, by J. L. Reagan. Paratypes; three males taken by R. B. Eads from skins of *Cratogeomys castanops* collected by W. F. Blair from Armstrong County, Texas, July 9, 1947; a male and female collected by personnel of the U. S. Public Health

Service Plague Investigation Service in Hartley County, Texas, June 1944, from *Thomomys* sp. probably *T. bottae;* thirteen female and nine male specimens taken from seven *Cratogeomys castanops* by J. L. Reagan and Sam Awolt during June 1948 in Presidio County, Texas.

This flea appears to be most closely related to *D. neomexicana* Prince. However, as can be noted from the illustrations, both sexes readily can be separated by the modified abdominal segments. The movable finger of *D. ncomexicana* is about four times as long as the process of the clasper and about twice as long in *D. percernis;* the two movable fingers are differently shaped and bear different numbers of bristles.

The specimens of *Cratogeomys castanops* (Baird) were identified by W. F. Blair. The identification of the *Thomomys* was made by F. M. Prince. As *T. bottae* is the only species of this genus recorded from West Texas by Taylor and Davis (1947), it is presumed that this is the host.

The holotype and allotype have been deposited in the collection of the U. S. National Museum, Washington, D. C. Paratypes have been sent to F. M. Prince, U. S. Public Health Service Plague Investigation Station, San Francisco, California; Major Robert Traub, Army Medical Center, Washington, D. C.; and Dr. W. L. Jellison, Rocky Mountain Laboratory, U. S. Public Health Service.

REFERENCES

PRINCE, FRANK M. 1945   Descriptions of Three New Species of *Dactylopsylla* Jordan and One New Subspecies of *Foxella* Wagner, with Records of Other Species In The Genera. Can. Ent. **77:** 15.

TAYLOR, W. P. AND DAVIS, W. B. 1947   The Mammals of Texas. Game, Fish and Oyster Commission Bul. No. 27, 79 p.

EXPLANATION OF PLATES I AND II

Dactylopsylla percernis: 1, head of male; 2, female VII sternite; 3, modified abdominal segments of male; 4, VII sternite of male; 5, clasper and movable finger of the clasper of male.

Dactylopsylla neomexicana: 6, VII sternite of female; 7, modified abdominal segments of male.
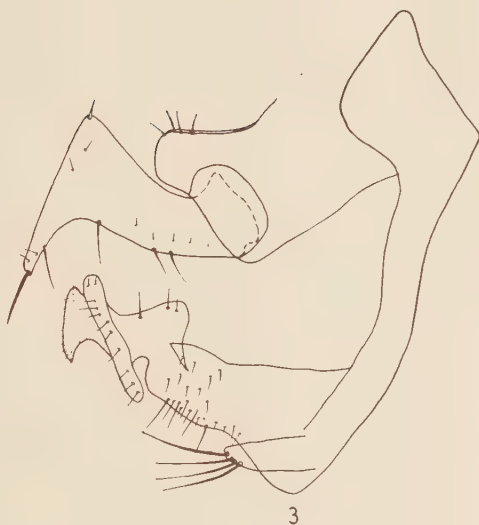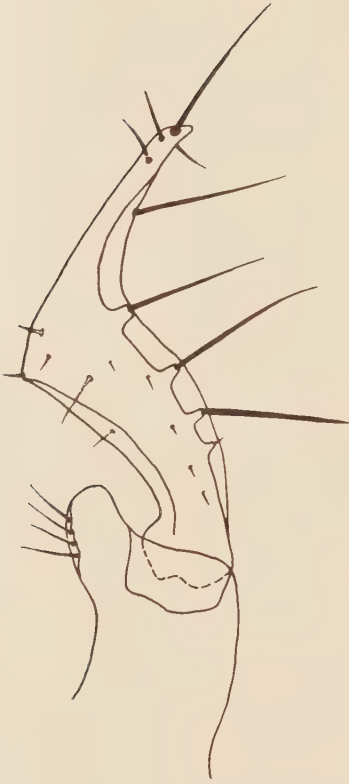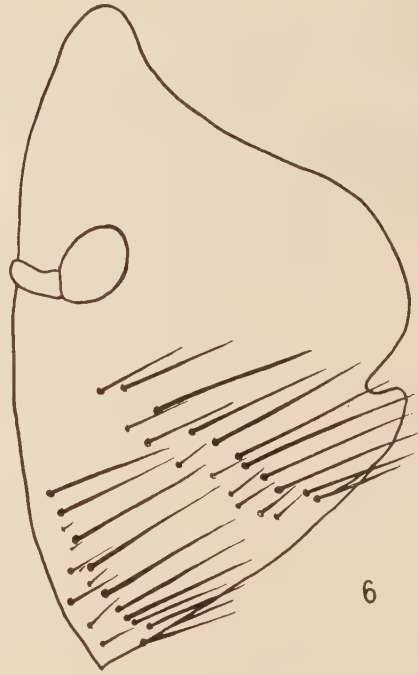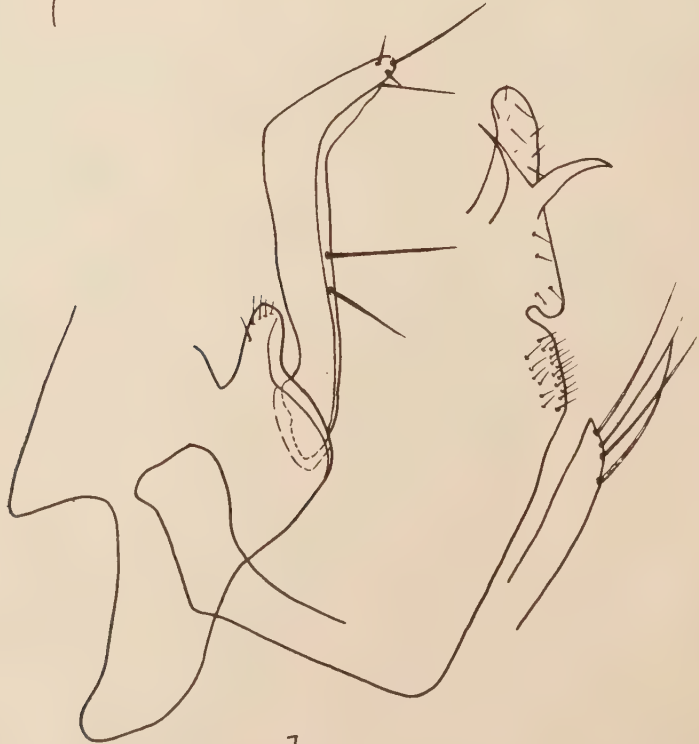
PLATE I

1

2

3

4

PLATE II

NITROFURAZONE IN THE MEDICATION OF AVIAN COCCIDIOSIS

Paul D. Harwood and Dorothy I. Stunz

Dr. Hess and Clark, Inc., Ashland, Ohio

The word, nitrofurazone, was selected to simplify the chemical name of 5-nitro-2-furaldehyde semicarbazone which was introduced recently into human medicine by Eaton Laboratories, Inc. Its pronounced *in vivo* activity against several species of bacteria and against at least one protozoan (*Trypanosoma equiperdum*) suggested that the drug should be tested against *Eimeria tenella* in chickens.

HISTORICAL

A thorough review of the literature dealing with the therapy of coccidiosis is not attempted. Although our incomplete files list many more than one hundred materials which have been tested against this disease, not more than 6 of these are of any practical significance. Many chemicals proved ineffective, others, although effective were too toxic and a few, though effective, remain mere laboratory chemicals that are not available in commercial quantities as yet. Only a few of the more important chemicals used against coccidiosis are mentioned at this time, and for each of these only one or two recent references are cited.

Sulfur was the first of the truly effective drugs to be used. Goff (1942), has given one of the more recent reports on the use of sulfur. Unfortunately sulfur must be fed before the birds acquire coccidiosis to be fully effective, and it is not without troublesome side effects. Addition of charcoal and urea have not proved satisfactory in prevention of these unfavorable reactions.

The use of borax in coccidiosis which was described by Hardcastle and Foster (1944), and Kay (1947), has not proved feasible because effective concentrations retard growth.

Probably the sulfa-compounds have been studied most extensively as coccidiostatics, and these compounds are used widely in commercial control of the disease although they are very expensive. Seeger (1947), Swales (1947) and Peterson (1948) have discussed the practical use of the sulfa drugs in recent papers. Waletzky and Hughes (1946) have discussed from a more theoretical standpoint the characteristics of these sulfa drugs. According to current practices 0.5 to 1 per cent of sulfaguanidine, 0.4 per cent of sulfamethazine, 0.4 per cent of sulfamerazine, and 0.1 per cent sulfaquinoxaline are administered to chickens in an all-mash feed. All these treatments, except 0.5 per cent of the first drug prevent death losses if given not later than 3 days after infection. Medication 4 days after infection is unreliable, although possibly of benefit in some instances. Sodium salts of some of these compounds may be administered in the drinking water.

Aryl arsonic acids, as described by Morehouse and Mayfield (1946), are effective if given before the day of infection. This drug is usually administered in small concentration in the drinking water.

The bacteriostatic action of nitrofurazone and of related compounds was first described by Dodd and Stillman (1944), and the chemistry of the compound by

Stillman and Scott (1945). Information on the activity of the compound *in vivo* against experimental infections of mice with *Streptococcus* and *Trypanosoma equiperdum* were presented by Dodd (1946). The pharmacology of the drug was described by Krantz and Evans (1945), and its use in dermatology by several authors among whom Downing, Hanson and Lamb (1947) may be cited as representative.

MATERIAL AND METHODS

Day-old chicks of various breeds were purchased from commercial hatcheries, and brooded in wire-floored, electrically heated, battery brooders. At one or two weeks of age the chicks were banded, weighed individually and separated into groups of approximately 20 birds each. These groups were carefully balanced by weight. The birds were weighed individually every four days, and eight days after banding they were infected *per oram* individually with approximately fifty thousand sporulated oocysts of *Eimeria tenella*. The last weight was taken eleven days after infection (3 days after the previous weighing) when the experiments were usually terminated.

The chief criteria of effectiveness throughout these experiments were the deaths in control and treated groups. However, additional evidence was obtained from weights and the more important of these are recorded in tabular form in this article.

Throughout these experiments the birds were given as their sole diet an all-mash feed having the following formula: yellow corn meal, 800 lbs.; middlings, 300 lbs.; bran, 200 lbs.; ground oats, 160 lbs.; meat scraps, 140 lbs.; soybean oil meal, 240 lbs.; alfalfa leaf meal, (17% dehydrated), 100 lbs.; cod liver oil, (800-D), 2.5 lbs.; and a commercial mineral mixture, 60 lbs.

Nitrofurazone was kindly furnished to us by Eaton Laboratories. It consisted of two grades: (1) the pharmaceutical grade, which is a light yellow powder of very high purity; (2) the veterinary grade, which is more greyish in color and contains a very small amount of extraneous matter. The grade employed is recorded for each test, but little difference in biological activity was noted.

Chemically, nitrofurazone is 5-nitro-2-furaldehyde semicarbazone and has the following structural formula:

$$O_2N-C \underset{\diagdown O \diagup}{\overset{\displaystyle HC-CH}{\underset{\displaystyle ||\quad\ ||}{}}} C-CHNNHCONH_2.$$

It is rather insoluble in water. It crystallizes as yellow needles; the melting point is not sharp, but the material liquifies with some tarry decomposition in the range 227–241° C.

The drug was administered to the chickens in an all-mash feed, or in drinking water, at several different concentrations and at different times relative to the day of infection.

EXPERIMENTAL RESULTS

Experiment 1:

In a preliminary test, fifteen New Hampshire chicks, hatched November 11, 1946, were given a medicated mash containing one part of nitrofurazone, pharmaceutical grade, to 1,100 parts of mash on January 6, 1947. Four days later the birds were weighed and given by mouth approximately 100,000 oocysts of *Eimeria tenella*. At this time, the average weight of the birds had fallen from 526.2 grams

TABLE 1.—*Efficacy of nitrofurazone (pharmaceutical grade) in the therapy of experimental coccidiosis due to* Eimeria tenella

Note: Rhode Island Red Cockerels which were hatched May 22, 1947, were matched by weight and 20 birds distributed to each pen. Average weight of each pen on June 2 was 78.7 grams. Each bird was given 50,000 oocysts on June 10. Medication continued to end of test.

Pen No.	Parts of feed to one part of drug	Date treatment commenced	Mean weight of birds in grams			Number of Deaths
			June 10	June 18	June 21	
1	6,000	June 8	141.6	193.6	222.1	0
2	4,500	June 10	143.9	192.6	208.5	0
3	4,500	June 12	136.5	191.4	207.5	0
4	Infected controls	......	136.5	140.0	171.5	18

to 486.1 grams, presumably because of drug toxicity. Therefore, the remaining medicated mash was mixed with an equal quantity of unmedicated feed, thereby reducing the drug concentration to one to 2,200. On this reduced medication the birds began to gain weight but not at the normal rate. Also the birds seemed less alert than usual. Not one of the medicated birds developed any symptom of coccidiosis, but five out of 15 similar controls died of the disease. The surviving controls were severely affected and lost 30 grams on the average.

Experiment 2:

In a second preliminary test, nitrofurazone, pharmaceutical grade, was mixed one to 4,500 in an all-mash feed and given to 25 Rhode Island Red cockerels on May 6, 1947. The cockerels were hatched April 27, and were infected with 50,000 oocysts on May 9. There were no deaths from the disease among the treated birds and only a few bloody droppings on the fifth and sixth days after infection. Fourteen out of 25 similar controls died of the infection. Comparison of weight-gains suggested that the nitrofurazone at this concentration was slightly toxic since the treated birds gained only 20.4 grams on the average from May 9 to May 13, while the control birds gained 27.4 grams. However, the difference in gain may have been due to unpalatability of the medicated mash or, quite likely, to some chance factors. There was no noticeable effect of the drug upon the behavior of the birds.

Experiments 3 to 9:

The results of these tests are presented in detail in tables 1 to 7.

Toxicity:

The results of administering large doses of nitrofurazone to cockerels are presented in table 8. The symptoms displayed by these birds were conspicuous. They

TABLE 2.—*Efficacy of nitrofurazone (pharmaceutical grade) in the therapy of experimental coccidiosis due to* Eimeria tenella

Note: Each pen contained 22 new Hampshire chicks of mixed sexes that were hatched July 6, 1947. The chicks of each pen were matched by weight. On July 21, the mean weight of each pen was 101.3 grams. Each bird was given 50,000 sporulated oocysts July 24. All medication terminated on August 1.

Pen No.	Parts of feed to one part of drug	Date treatment commenced	Mean weight of birds in grams			Number of Deaths
			July 24	Aug. 1	Aug. 4	
1	9,000	July 22	125.7	195.3	211.1	0
2	6,000	July 26	128.0	191.2	210.4	0
3	6,000	July 27	126.5	138.8	177.8	9
4	9,000	July 26	128.3	197.7	225.7	0
5	Infected controls		129.8	140.3	167.5	16
6	Uninfected-controls		128.5	206.0	243.4	0

TABLE 3.—*The relationship between concentration of nitrofurazone and the time of commencing treatment*

Note : Each pen contained 21 White Leghorn chicks of mixed sexes that were hatched September 22, 1947. On September 29, the birds were matched by weight. The mean weight for each pen on that date was 68.9 grams. Each bird was given 50,000 sporulated oocysts October 7. Treatment was terminated October 16 in all pens.

Pen No.	Parts of feed to one part of drug	Date treatment commenced	Mean weight of birds in grams			Number of Deaths
			Oct. 7	Oct. 15	Oct. 18	
1	9,000*	Oct. 7	110.2	163.0	189.3	0
2	9,000	Oct. 7	114.9	169.4	197.8	1**
3	11,250	Oct. 7	110.4	160.3	186.6	3
4	15,000	Oct. 7	114.1	154.3	175.3	3
5	22,500	Oct. 7	115.2	153.2	177.1	11**
6	45,000	Oct. 7	115.6	131.9	151.5	12
7	3,000	Oct. 10	116.7	149.5	175.2	0
8	Infected control		115.6	124.3	147.0	18

* Nitrofurazone of veterinary grade was used in Pen 1 ; the pharmaceutical grade in all other pens.
** The caretaker inadvertently destroyed the body of one bird from this pen between October 11 and October 15. As this was the period of the epidemic, it is assumed this bird died of coccidiosis.

ate practically nothing the rest of the day after dosing. Beginning about 4 hours after treatment, individual cockerels showed pronounced symptoms of excitement, although the birds seemed generally to be markedly depressed. During excitement, the birds squawked loudly, often supported themselves on the front of the breast and the extended, rigid legs. Again they squatted with the head thrown over back, neck and wings extended, the latter shaken by severe tremors. At other times the chicken flung itself about kicking and flopping its wings like a bird newly decapitated. These fits lasted for several minutes. Afterwards the affected bird seemed profoundly depressed and exhausted. These seizures continued as late as eleven o'clock on the night of dosing, but had stopped completely by 8 o'clock the next morning. The first deaths occurred between 11 P.M. September 16 and 8 A.M. September 17. The surviving birds continued to be markedly depressed for 3 days after dosing. The majority of deaths occurred on the second and third days

TABLE 4.—*Efficacy of 0.033 per cent nitrofurazone administered in the feed for 24 hours compared with lower dosage rates administered for longer periods in the control of coccidiosis due to* Eimeria tenella

Note : Each pen contained 30 White Leghorn chicks of mixed sexes which were hatched October 27, 1947. Each pen contained 2 groups of 15 birds which were matched by weight. The mean weight for each group was 67.4 grams on November 3. Fifteen birds in each cage were infected with 50,000 oocysts on November 11, the remaining 15 served as uninfected controls receiving the medicated ration. Weights of infected birds are given on the upper line ; of uninfected, on the lower line.

Pen No.	Parts of feed to one part of drug	Date dosing commenced	Duration of dosing (days)	Mean weight of birds in grams			Number of Deaths
				Nov. 11	Nov. 19	Nov. 22	
1	3,000*	Nov. 11	1	112.9	157.5	197.5	13
				116.4	182.7	209.7	0
2	3,000*	Nov. 12	1	111.6	151.7	186.3	12
				116.8	188.3	218.4	0
3	3,000	Nov. 13	1	123.6	164.5	190.5	13
				117.3	179.9	207.5	0
4	3,000*	Nov. 14	1	113.9	145.0	184.5	13
				111.9	174.5	201.8	0
5	3,000*	Nov. 15	1	109.3	135.0	129.0	14
				112.3	178.7	204.0	0
6	3,000	Nov. 14	1	112.5	134.0	155.2	9
	9,000	Nov. 15	4	111.3	153.0	171.9	0
7	15,000	Nov. 11	8	110.5	170.5	195.1	6
				115.1	185.9	212.9	0
8	9,000	Nov. 11	8	111.5	179.5	206.3	0
				111.4	176.1	202.7	0
9	Controls	Not dosed	. .	116.1	118.0	161.0	14
				108.9	177.7	205.2	0

* Veterinary grade nitrofurazone was used in these cages ; pharmaceutical grade in the others.

TABLE 5.—*Efficacy of nitrofurazone when administered in the presence of dried milk and alfalfa leaf meal or dissolved in water*

Note : Each pen contained 15 White Leghorn chicks of mixed sexes which were used as uninfected controls in the experiment detailed in Table 4. Each bird was infected with 100,000 oocysts on November 25, 1947. Medication began on November 25 and was continued for 9 days, except Pen 5 in which medication began on November 25 and continued for 8 days.

Pen No.	Vehicle of administration of drug	Parts of feed or water to one part of drug	Mean weight of birds in grams			Number of Deaths
			Nov. 25	Dec. 3	Dec. 6	
1	Feed	9,000*	242.7	319.3	349.6	0
2	Controls	.....	254.2	281.8	302.2	7
3	Feed	9,000*	227.8	285.2	320.4	1
4	Controls	.....	230.5	249.4	293.2	10
5	Water	20,000**	224.3	214.0	239.0	12
6	Water	18,000**	244.1	286.6	326.6	7
7	Water	22,000**	253.3	280.4	333.4	7
8	Uninfected controls	.....	242.9	347.1	381.5	0

* Equal parts of alfalfa leaf meal and dried milk were mixed and to 90 parts of this premixture one part of nitrofurazone was added. One per cent of this premixture was used to make the medicated feed.
** A solution of 0.2 per cent nitrofurazone in polyethylene glycols was diluted to make the solution used as drinking water in Pen 5. The solutions used in Pens 6 and 7 were made without use of auxillary solvents.

after dosing, but some birds died as late as September 22 and 23. The depression began to lessen about the fourth day after dosing and some of the birds were gaining weight by September 24.

All birds which died of the effect of dosing were necropsied. There was a marked catarrhal inflammation in all digestive organs except the gizzard. The liver and kidneys were intensely congested, and in many cases hemorrhages were present in the kidneys. Pectechiae were frequently found in the thymus and less frequently in other parts of the body. Also hemorrhages were frequently found in the occipital, parietal, and squamosal bones of the skull. These hemorrhages were unilateral sometimes and confined to one bone or in other birds, bilateral and in all three bones. The meninges over the cerebellum were congested, but those over the cerebrum seemed more nearly normal.

Sections for microscopical examination were cut from selected tissues. While

TABLE 6.—*The effect of various vehicles upon the efficiency of nitrofurazone as a coccidiostatic*

Note : The chickens were White Leghorn cockerels that were hatched January 5, 1948. On January 15 they were divided into 14 groups of 15 chickens each. The mean weight of each group varied from 64.0 to 65.5 grams at that time. Two groups were confined to each pen ; those of the principal group were given 50,000 sporulated oocysts of *Eimeria tenella* on January 23, 1948, and medicated feed which was first placed in the pens 53 hours after infection was fed for 3 days ; the uninfected group served as controls receiving the same treatment. Weights of infected birds are given on the upper line ; of uninfected, on the lower line.

Pen No.	Vehicle for administration of drug	Parts of feed or water to one part of drug	Mean weight of birds in grams			Number of Deaths
			Jan. 23	Jan. 31	Feb. 3	
1	1% Soybean meal in feed	9,000	103.3	144.1	169.0	1
			98.7	154.1	180.3	1**
2	1% Kaolin in feed	9,000	99.1	144.8	167.7	0
			97.5	147.3	176.5	0
3	1% Iron oxide in feed	9,000	107.1	157.9	181.7	1
			104.1	165.2	194.0	0
4	Feed	9,000*	98.6	147.2	174.1	1
			100.1	149.1	174.9	0
5	Controls	.....	98.5	127.3	134.3	12
			102.1	149.5	177.3	0
6	Water in paraffined trough	15,000	103.8	149.1	176.9	1**
			98.1	150.1	181.1	2**
7	Water	9,000	99.7	137.7	158.3	8**
			95.5	132.4	152.8	0

* Nitrofurazone was given to this pen on January 17 ; the treatment was continuous throughout the duration of this test.
** These birds proved lightly infected with pullorum disease when received. Consequently, deaths which were not due to coccidiosis occurred in the following groups. Pen 1, group II, one death ; pen 6, all three deaths ; pen 7, group I, one death.

TABLE 7.—*Relationship between time of infection and time of beginning treatment of experimental coccidiosis with nitrofurazone*

NOTE: These birds are the uninfected controls of Table 1. They were retained in the same pens, and 2 pens not reported in Table 6 are included here. They were each given 100,000 oocysts of *Eimeria tenella* on February 3, 1948. A pre-mix containing 5 per cent iron oxide, 93.9 per cent soybean oil meal and 1.1 per cent nitrofurazone was made and 1 per cent of this pre-mix added to the feed. The final feed-drug ratio was 9,000 to 1. Except for Pen 4, medication was terminated on February 10.

Pen No.	Number of Birds	Hours elapsing between infection and start of medication	Mean weight of birds in grams				Number of Deaths
			Feb. 3	Feb. 7	Feb. 11	Feb. 14	
1	14	27	185.4	223.6	262.9	293.4	0
2	15	56	176.5	218.0	250.9	294.5	1
3	15	56	194.0	229.1	261.1	284.8	0
4	14	*	173.1	205.6	251.9	301.0	2**
5	15	Control	177.3	214.9	232.7	284.3	9
6	13	64	181.1	220.4	238.4	286.4	2
7	15	64	167.2	197.2	236.3	293.3	5
8	14	77	151.9	176.4	189.7	251.0	12
9	15	77	152.8	183.9	203.1	264.8	5

* Pen 4, which was first given 1 to 9,000 nitrofurazone on January 17, was continued on this medication until the experiment was terminated on February 17.
** One of these 2 birds died of causes not related to the experimental infection or the treatment.

no claim is made for completeness in regard to the pathological study, some of the observations may be of interest. The gastro-intestinal tract exhibited a moderate catarrhal enteritis. There was moderate venous congestion in this tract, as well as in the cerebellum, and thymus. The liver and lungs exhibited intense passive congestion. The kidneys showed very intense active congestion. The nuclei in the convoluted tubules stained faintly and the cytoplasm of these cells exhibited advanced cloudy swelling and other degenerative changes.

The most significant pathology observed either grossly or microscopically seemed to be in the kidney. However, the generalized enteritis may have been important. At this time the correlation of symptoms and pathology is impossible. It is questionable if the periods of excitation can be related causally to either the enteritis or the nephritis. This type of motor stimulation suggests possible injury to the central nervous system, particularly the cerebellum, but the writers are not sufficiently experienced in neuro-pathology to assess accurately the significance of the observed congestion, which seemed rather trivial.

DISCUSSION

The quantity of nitrofurazone necessary to control losses in experimental coccidiosis of fowls due to *Eimeria tenella* is surprisingly small. The results obtained in pens 1 and 4, table 2, and in subsequent tests indicate that one part of nitrofurazone to 9,000 parts of an all-mash feed is effective in this condition. Six of 235 birds, or 2.5 per cent, given the drug at that level in the feed died of coccidiosis.

TABLE 8.—*The acute, toxic dose of nitrofurazone for Rhode Island Red cockerels*

Note: These cockerels which were hatched July 6, 1947, were dosed in groups of 12 cockerels each. The birds were confined to individual cages after dosing.

Group No.	Grade of Drug	Dosage Rate (mg./kg.)	Date of Dosing	Mean weight of birds (grams)	Number of Deaths
1	Pharmaceutical	200	Sept. 16	1,054.3	8
2	Veterinary	200	Sept. 16	1,166.3	9
3	Pharmaceutical	300	Sept. 16	1,010.0	10
4	Veterinary	300	Sept. 16	1,114.9	12
5	Pharmaceutical	400	Sept. 16	1,072.8	12
6	Veterinary	400	Sept. 16	1,046.8	12
7	Pharmaceutical	150	Oct. 10	1,802.0	5
8	Veterinary	150	Oct. 10	1,656.0	2

On the other hand 67 of 88 control birds, or 76 per cent, died of similar infections. Since the experimental infections were unquestionably heavier than those of most natural infections, the one to 9,000 level of the drug seems high enough for practical purposes. Higher concentrations might prove less beneficial since the object of coccidiosis medication is not to destroy the parasite, but only to reduce losses until host-resistance has time to develop. Therefore, the one to 9,000 level seems to be the best compromise.

To test the effect of nitrofurazone therapy upon the development of hostal resistance, all birds used in collecting the data reported in table 2 were dosed again with 200,000 infective oocysts each on August 9, 1947. Five of the twenty-two birds from the uninfected controls died as a result of this infection. No other bird showed any ill effects.

The effects of higher dilutions of the drug are presented in table 3, where it may be seen that one part of the drug in 22,500 parts of feed, or even in 45,000 parts seems to have some beneficial effect on the course of the disease. Quantitatively speaking, therefore, nitrofurazone is far more effective than chemicals used heretofore against *Eimeria tenella* infection in chickens.

The time of treatment in relation to the date of infection is likewise a matter of some importance. As shown in table 7, nitrofurazone is fully effective if given no later than 56 hours after infection. At 64 hours after infection, some deaths occur; at 77 hours, no protective action is discernible. In this respect nitrofurazone is more satisfactory than sulfur (Goff, 1942) or the arsonic acids (Morehouse and Mayfield, 1946), approximately as satisfactory as sulfaguanidine (Wehr and Farr, 1945); but less satisfactory than sulfamethazine (Swales, 1947).

Apparently the feed is the only satisfactory vehicle for the administration of nitrofurazone to fowls. Since the compound is active in very small doses, it is theoretically possible to administer it in effective quantities in the drinking water, although it is but slightly soluble in water. However, tests reported in table 5 (pens 5, 6 and 7) and table 6 (pens 6 and 7) indicate that it should be given in the feed or in paraffin coated troughs for best results. The uncoated metal troughs containing a solution of nitrofurazone showed excessive amounts of rusty sediment after 24 hours. Possibly reduction of the nitrofurazone incidental to the oxidation of the iron is responsible for the lowered efficacy when solutions are exhibited in uncoated metal troughs.

The necessary duration of dosing was explored in experiment 6 (table 4). One day of treatment was not sufficient, regardless of the development of the parasite. However, the data presented in table 6 indicate that 3 days of dosing may be adequate.

The ratio between the effective daily dose and the median lethal dose is approximately one to ten. This relationship is approximately the same as the similar ratio for the more effective sulfa-compounds. However, in the matter of toxicity nitrofurazone seems to possess one advantage over sulfamerazine, and presumably sulfamethazine. Farr and Jaquette (1947) found that therapeutic doses of sulfamerazine retarded growth, and caused necrotic areas in the spleen when administered for one week in the feed. Comparison of the weight gains of pens 4 and 5 in tables 6 and 7 indicates that two weeks continuous feeding of nitrofurazone at the therapeutic

level does not retard growth.   After four weeks of feeding this drug retardation is so slight, it is questionable if it is of any significance.

SUMMARY AND CONCLUSIONS

1. Nitrofurazone proved effective in extremely small doses for the control of experimental coccidiosis produced by *Eimeria tenella.*

2. One part of the drug in 9,000 parts of feed was sufficient to prevent losses if given not later than 56 hours after infection.   At this level the drug did not interfere with the development of immunity.

3. Feed medicated at the effective level did not cause any growth retardation. Likewise medicated feed was readily accepted by the chickens.

4. Nitrofurazone is not effective when dissolved in water and placed in metal troughs.   Paraffined troughs apparently prevent the deterioration of the drug.

5. The median lethal dose of nitrofurazone lies somewhere between 150 and 200 mgm. per kilo according to limited tests.

LITERATURE CITED

DODD, M. C. 1946 The chemotherapeutic properties of 5-nitro-2-furaldehyde semicarbazone (Furacin). J. Pharm. Exper. Therap. **86:** 311–323.
DODD, M. C. AND STILLMAN, W. B. 1944 The *in vitro* bacteriostatic action of some simple furan derivatives. J. Pharm. Exper. Therap. **82:** 11.
DOWNING, J. G., HANSON, M. C. AND LAMB, M. 1947 Use of 5-nitro-2-furaldehyde semicarbazone in dermatology. J. Am. Med. Assoc. **133:** 299–306.
FARR, M. M. AND JAQUETTE, D. S. 1947 The toxicity of sulfamerazine to chickens. Am. J. Vet. Res. **8:** 216–220.
GOFF, O. F. 1942 Period of protection afforded by sulphur in prevention of *Eimeria tenella* infections. Poult. Sci. **21:** 155–160.
HARDCASTLE, A. B. AND FOSTER, A. O. 1944 Notes on a protective action of borax and related compounds in cecal coccidiosis of poultry. Proc. Helminth. Soc. Washington. **11:** 60–64.
KAY, M. W. 1947 Medication of cecal coccidiosis of chicks. J. Am. Vet. Med. Assoc. **110:** 20–24.
KRANTZ, J. C., JR. AND EVANS, W. E., JR. 1945 A contribution to the pharmacology of 5-nitro-2-furaldehyde semicarbazone. J. Pharm. Exper. Therap. **85:** 324–331.
MOREHOUSE, N. F. AND MAYFIELD, O. J. 1946 The effect of some aryl arsonic acids on experimental coccidiosis infection in chickens. J. Parasit. **32:** 20–24.
PETERSON, E. H. 1948 The effect of sulfaquinoxaline medication on *Eimeria tenella* infection in chickens. Am. J. Vet. Res. **9:** 77–84.
SEEGER, K. C. 1947 Flock *Eimeria tenella* inoculation followed by sulfaguanidine or sulfamethazine treatment as a method of controlling caecal coccidiosis. Poult. Sci. **26:** 554–555.
STILLMAN, W. B. AND SCOTT, A. B. 1945 Series of nitrofuran compounds. U. S. Patent 2,416,234.
SWALES, W. E. 1947 New methods of controlling caecal coccidiosis in chicks. Canad. J. Comp. Med. **11:** 5–10.
WALETZKY, E. AND HUGHES, C. O. 1946 The relative activity of sulfanilamides and other compounds in avian coccidiosis (*Eimeria tenella*). Am. J. Vet. Res. **7:** 365–373.
WEHR, E. E. AND FARR, M. M. 1945. Effect of sulfaguanidine on the course of infection in chickens with *Eimeria tenella.* J. Parasit. **31:** 359–365.

SOME CONDITION CHARACTERISTICS OF A YELLOW PERCH POPULATION HEAVILY PARASITIZED BY *CLINOSTOMUM MARGINATUM*[1]

A. M. ELLIOTT AND L. R. RUSSERT

Department of Zoology, University of Michigan, Ann Arbor, Michigan

The effects of parasitism on the health of fish hosts are virtually unknown except for obvious instances in which the parasites cause death (Davis, 1946), destroy reproductive potential (Hunninen, 1935), or bring about blindness (Ferguson and Hayford, 1941, La Rue *et al.*, 1926). The purpose of this investigation was to explore some less evident relationships of one common fish parasite (*Clinostomum marginatum*) to a single species of fish host (yellow perch, *Perca flavescens*), in a small lake.

Clinostomum marginatum, the parasite involved in this study, commonly infects the yellow perch as a second intermediate host where it encysts in the integument and musculature as a metacercaria. The adult stage is attained in the oral cavity of the great blue heron, *Ardea herodias,* eggs being shed into the water as the bird host feeds; miracidial stages enter the appropriate snail species, (*Helisoma campanulatum* and *H. antrosum*) ; and the emergent cercariae in turn penetrate the tissues of the second intermediate host, often the yellow perch.

Very nearly all of the perch examined in this study were infected with the metacercarial stage of *Clinostomum marginatum.* The host sample was composed of some 2,200 specimens from an ostensibly grubby population in Buck Lake, a 40-acre body of water in northern Minnesota.[2] These fish were obtained in a single seining operation in August, 1940, by the senior author. The sample is random within the limits of the selectivity of the collection method which involved the use of a 300-foot 1-inch mesh seine ; no particular attempt was made to obtain or save only fish of a particular size, sex, or other characteristic. Initial preservation was in formalin.

During the year following collection, each fish was treated as follows: standard length was measured to the nearest millimeter ; preserved weight was taken to the nearest 0.1 gram ; number of parasites visible to the naked eye on dissection was recorded ; contents of the stomach were analyzed ; and age was determined from a scale sample.

The autopsy for parasites in each perch was made by cutting thin cross-section slices from the fish, beginning at the tail region and progressing serially forward. The number of parasites visible to the naked eye in each section was counted.

In order to test for a relationship between relative robustness of a host and the number of parasites it harbored, the conventional coefficient of condition (K) used

by fishery workers was calculated for each fish.   This factor has been of considerable use to fishery biologists in comparing the condition (i.e. the degree of relative well-being) of a fish species in various environments.

The formula used for computing the coefficient of condition is an expression of the Cube Law:

$$K = 100,000 \ W/L^3 ; \text{ or } K = W \times 10^5/L^3$$

where K = the coefficient of condition;

W = the weight in grams;

L = standard length in millimeters; and

where the multiple 100,000 is employed merely to bring the value for K to a whole number plus a fraction, rather than to a less meaningful, small decimal fraction alone.
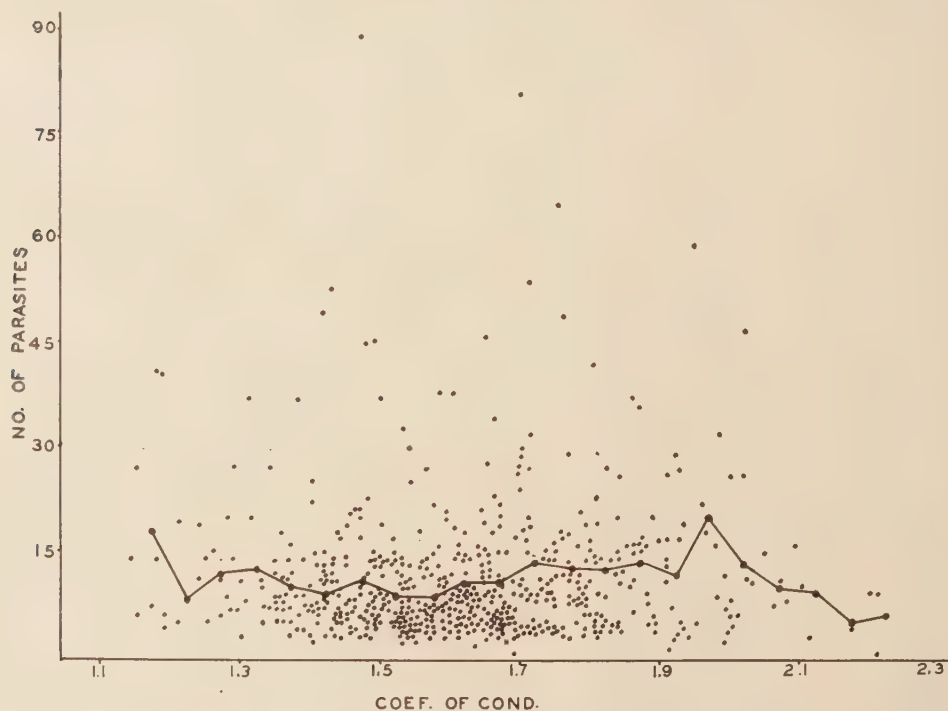

FIG. 1.   Scattergram of approximately 1200 1-year-old fish where number of parasites is plotted against coefficient of condition.   Solid line indicates mean number of parasites at different levels of K.

Thus, the higher the value of K for a fish, the better its condition and *vice-versa*.

Extensive computations and efforts to relate coefficient of condition and number of parasites yield negative evidence.   Scattergram analyses show no correlation of these two elements for the entire series of fish.   This conclusion is not changed when fish of each age group are considered separately.   (Fig. 1 is an exemplary scattergram for age group I, yearling fish).   The scattergram was drawn up with number of parasites (ordinate) plotted against the value of K (abscissa) for each fish.   A breakdown into age groups is requisite because of demonstrable differences in K in successive age groups which will be shown later.   In general, within each age group, fish with smaller coefficients of condition have no more or no fewer para-

sites than those with larger values of K. Only a very free distribution of both factors was evident (as in Fig. 1).

Failure of number of parasites to correlate with K may be due to certain characteristics of the coefficient of condition which are of interest *per se* in this perch population.
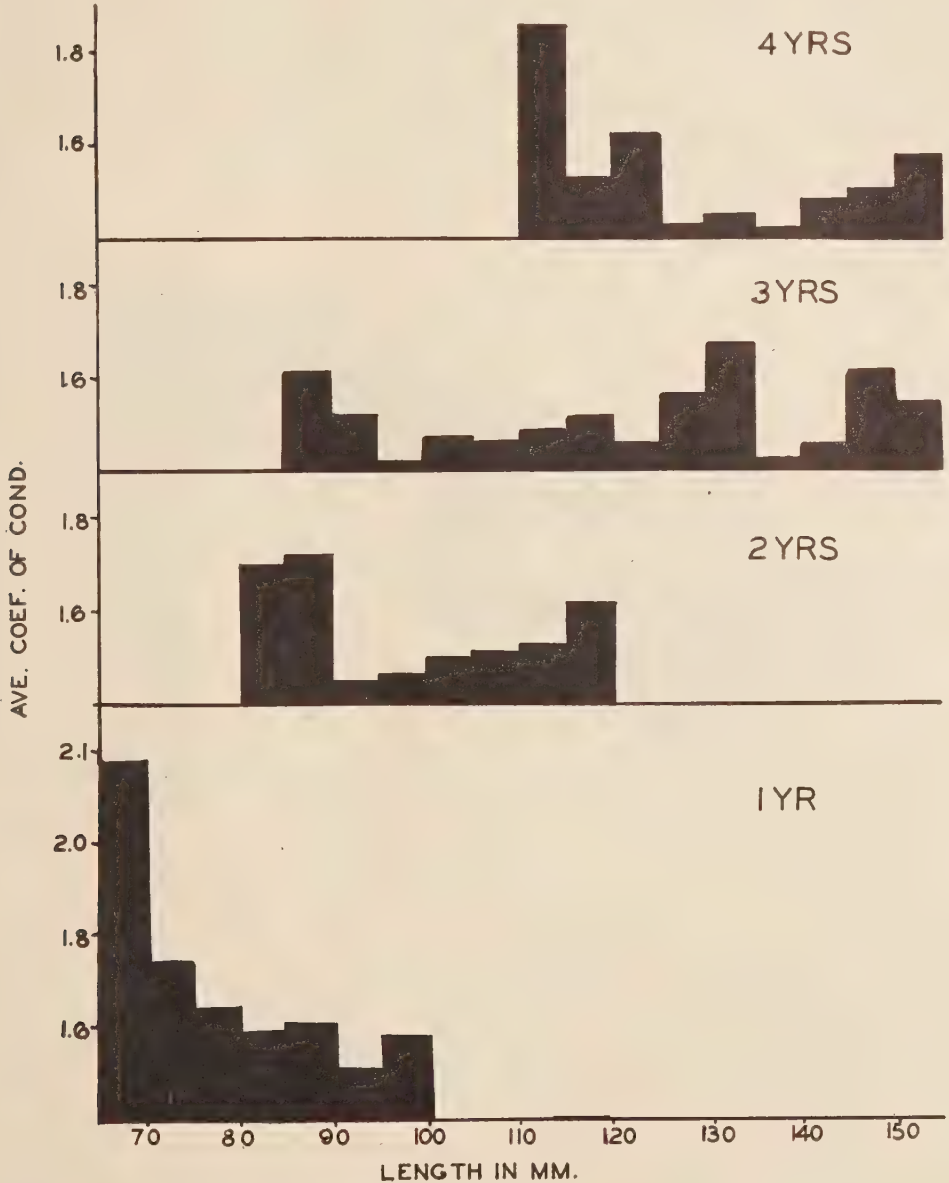

FIG. 2. Mean values of K at five-millimeter length intervals for age groups I through IV.

1. Within each age group mean values for K in successive five-millimeter length groups show a tendency to be higher for small fish, to be lower for fish of inter-

mediate length, and higher again in larger fish (Fig. 2). In other words, the shortest and longest fish appear to be more robust than ones nearer average length in the various age groups.

2. Compared with the average value of K for perch from the entire state of Minnesota, that for this fish in Buck Lake is very low. The best comparison is precluded, however, since the two sets of data are based on different weights. The Buck Lake weights were taken on preserved fish, while the averages of Minnesota fish in general are based on fresh weights. Dr. R. E. Johnson of the Minnesota Department of Conservation (1948, personal communication), reports that experiments are now underway for the purpose of determining just what happens to lengths and weights of fish preserved in formalin for various lengths of time. There
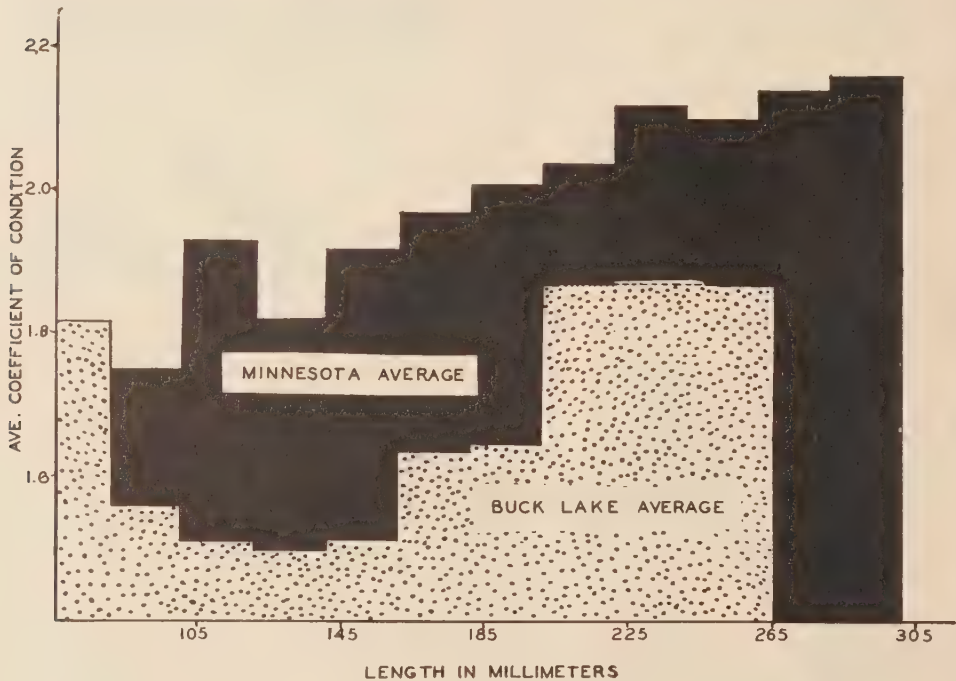

Fig. 3. Comparison of mean K values at various length intervals in Minnesota and Buck Lake perch.

have been some contradictory indications of fish taking on weight (Johnson, 1948, personal communication) or losing it (Shetter, 1936) in the course of preservation. Shetter found shrinkage to occur in the brook trout, *Salvelinus fontinalis,* through rigor mortis as well as through subsequent preservation. He found it possible to work out correction factors to compensate for these changes. The development of a correction factor application to our data will of course make them more meaningful.

3. The mean coefficient of condition for each twenty-millimeter size group of the entire population was compared with similar data for Minnesota perch in general (Fig. 3). Both sets of data demonstrate a somewhat general increase in robustness with increase in size; however, Buck Lake fish are in poorer condition than the average for Minnesota. The exception of high values for K in small fish is again

to be noted here. Data for such small fish are lacking in the Minnesota general averages. The remaining lower values obtained may be due to the fact that Buck Lake fish were emaciated when captured, caused perhaps, by the high degree of in-
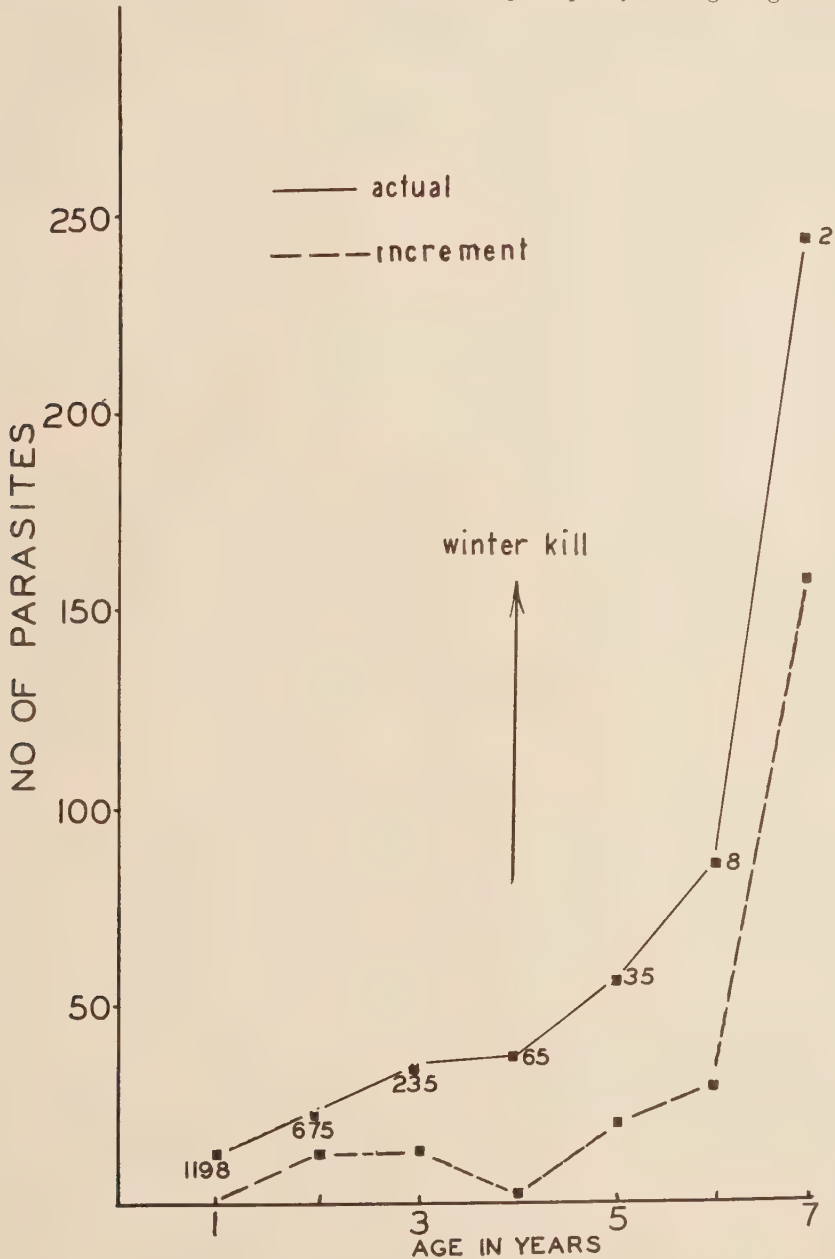

Fig. 4. Average number of parasites in Buck Lake perch at ages 1 through 7 years.

festation by *Clinostomum*; substantial weight loss during the period of their preservation would also be a factor, if it occurred. It must be admitted a condition of

over population combined with a short food supply might also account for an emaciated condition of the host.

Pursuing this analysis, a consistent increase is demonstrated in the mean number of parasites in each successive age group (Fig. 4). The annual increment in average number of parasites falls in the third and fourth years; it rises again from the fourth to the sixth. It should be noted that the picture presented by the first through third age groups, is better substantiated by numbers of perch than that for the fourth through the seventh year age groups—there being some two thousand fish in the first three categories, and only two hundred in the later ones. This phenomenon might be associated with a variation in the intensity of the infection of the snail population, and/or in the size of the snail population itself.

Another interesting coincidence may be seen here. Age group four, which is the first one to show a decline in mean number of parasites, was spawned in the spring of 1936. In the winter of 1936, there was a winter-kill (presumably due to winter anaerobiosis) in Buck Lake. This raises the question as to whether or not an infestation with *Clinostomum marginatum* may disturb the oxygen demand of perch so as to make them more susceptible to oxygen deficiencies and for other adverse conditions under the ice.

The coefficient of condition of this population when related to age of the fish, shows a steady decrease in the first three age groups. Beginning with the fourth and continuing through the seventh age groups, there is a steady rise in mean values for K. This may indicate that those presumably more hardy fish which survived the winter-kill of 1936, were further able to maintain a better condition because of the reduced number of fish in the population. Beckman (1940) showed experimentally that such a reduction in numbers of fish may increase the rate of their growth, presumably because of lessened competition for food.

<div align="center">DISCUSSION</div>

As many workers are well aware, comparatively little is understood about the relations of common fish parasites with their hosts, despite the fact that extensive faunal studies of fish hosts (Fischthal, 1947; Bangham and Venard, 1942; Nigrelli and Atz, 1943; Van Cleave and Mueller, 1934) have been made. La Rue *et al.* (1926); Palmer (1939); Hunter and Dalton (1939); Hunter and Hunter (1938); and others have made some studies of the more or less local reactions of fish hosts to some common strigeid parasites. Relatively little conclusive evidence has been presented by workers concerned with detecting a definite effect upon the general health of the host by a given parasite. Hubbs (1927) was rather certain that the debilitated condition of fish in a population of *Platygobio gracilis* could be attributed largely to their heavy tapeworm infection. Cross (1938) made a similar comparative study in which he found that fish with a relatively "heavy" parasite burden tended to be smaller and lighter than those with a "light" infection. Difficulty was encountered here in his calculations which were an approximate comparison of the size, as well as the number of parasites of different kinds in a fish, to the latter's size and weight. Hunter and Hunninen (1933) made the observation that river bass (*Micropterus dolomieu*) not parasitized with plerocercoid larvae of *Proteocephalus ambloplitis* were found to attain a greater weight than smallmouth bass of the same age groups taken from infected lakes. Hunninen (1935) had already

made note of the extensive damage accomplished by this parasite in the reproductive organs of its fish host.

More specifically related to these considerations is the work of Hunter and Hunter (1938) who attempted to detect the effect of *Crassiphiala ambloplitis* upon the weight of its hosts. They concluded that "a heavy infection of metacercariae of *C. ambloplitis* in young small-mouthed black bass, caused a statistically significant loss of weight when compared with the control fish."

In considering the effects on yellow perch of *C. marginatum,* Woodbury (unpublished thesis) contended that larger perch have a larger surface area and can thus acquire and house more parasites. A greater exposure to parasite infection may be a consequence of increased foraging activity, for a greater amount of food is needed as the size of the fish increases. Neither Woodbury's work nor the present investigation demonstrate any lethal effect of a heavy infection of metacercariae on young perch. Woodbury also attempted correlations between number of parasites and the condition factor, K, and found the former to have apparently no effect on the condition of the fish. Woodbury's specimens were mostly preserved in 10% formaldehyde before being examined, as were the fish in the Buck Lake collection.

Sex differences in K and in number of parasites were not considered here. Inasmuch as there is known to be a negligible difference in K between male and female perch except in the spring during spawning season (Jobes, 1948, personal communication), it seemed unnecessary to determine sex in subsequent examination of the fish. Woodbury found a consistently higher parasite index in female perch, but this he was able to attribute to their greater average length and weight (yielding the same proportionate K).

SUMMARY

1. The number of parasites (*Clinostomum marginatum*) infesting a population of some 2,200 perch shows no correlation with the coefficient of condition of these fish.

2. Only a very free distribution of both K values and numbers of parasites is discernible. Within each age group, the coefficient of condition is lowest in fish of intermediate size, higher for both smaller and larger fish.

3. The mean coefficient of condition decreases through the first three age groups, and then increases. The average number of parasites per fish shows a regular arithmetic increase with age and size of the fish. The increments are larger in the first three years, and smaller and irregular in later years.

4. The coincidental break noted with the four year groups of fish is thought to be associated with a winter-kill which occurred in Buck Lake following the season in which these fish were spawned. It suggests the hypothesis that older fish having a heavy infection with *Clinostomum marginatum* may have been more susceptible to anaerobiosis because of an increased oxygen demand, or because of a generally debilitated condition due to parasitism.

REFERENCES

BANGHAM, RALPH V. 1946 Parasites of northern Wisconsin fish. Trans. Wis. Acad. Sci., Arts and Letters **36**: 291–325.
———— and VENARD, CARL E. 1942 Studies on parasites of Reelfoot Lake fish. IV. Distribution studies and checklist of parasites. Jour. Tennessee Acad. Sci. **17**: 22–38.

BECKMAN, WILLIAM C. 1940 Increased growth rate of rock bass, *Ambloplites rupestris* (Rafinesque), following reduction in the density of the population. Trans. Amer. Fish. Soc. **70**: 143–148.

CARLANDER, KENNETH D. 1944 Notes on the coefficient of condition, K, of Minnesota fishes with an appended note on the coefficient of condition, C, of Minnesota fishes. Minn. Cons. Dept. Fisheries Res. Invest. Rept. No. **41**: 1–40. Revised June, 1944. (unpublished report)

CROSS, SAMUEL X. 1938 A study of the fish parasite relationships in the Trout Lake region of Wisconsin. Trans. Wis. Acad. Sci., Arts and Letters. **31**: 439–456.

DAVIS, H. S. 1946 Care and diseases of trout. U. S. Fish and Wildlife Service, Research Rept. **12**: 1–98.

FERGUSON, M. S., and HAYFORD, ROBERT A. 1941 The life history and control of an eye fluke. Prog. Fish Culturist. (54) : 1–13.

FISCHTHAL, JACOB H. 1947 Parasites of northwest Wisconsin fishes. I. The 1944 survey. Trans. Wis. Acad. Sci., Arts and Letters. **37**: 157–220. 1945.

HUBBS, CARL L. 1927 The related effects of a parasite on a fish. J. Parasit. **14**: 75–84.

HUNNINEN, A. V. 1935 Studies of fish parasites in the Delaware and Susquehanna watersheds. In : A Biological Survey of the Delaware and Susquehanna Watersheds. Suppl. 25th Ann. Rept. N. Y. Cons. Dept. 1935 : 237–245.

HUNTER, GEORGE W. III. 1936 Parasitism of fishes in the lower Hudson area. In : A Biological Survey of the Lower Hudson Watershed. Suppl. 26th Ann. Rept. N. Y. Cons. Dept. 1936. (11) : 264–273.

———1942 Studies on the parasites of freshwater fishes of Connecticut. In : A Fishery Survey of Important Connecticut Lakes. Conn. Geol. and Nat. Hist. Survey. Bull. **63**: 228–288.

——— and DALTON, H. CLARK. 1939 Studies on *Clinostomum*. V. The cyst of the yellow grub of fish (*Clinostomum marginatum*). Proc. Helminthol. Soc. Washington. **6**: 73–76.

——— and HAMILTON, JOHN MEACHAM. 1941 Studies on host-parasite reactions to larval parasites. IV. The cyst of *Uvulifer ambloplitis* (Hughes). Trans. Amer. Microsc. Soc. **60**: 498–507.

——— and HUNNINEN, A. V. 1933 Studies on the plerocercoid larva of the bass tapeworm, *Proteocephalus ambloplitis* (Leidy), in the small-mouthed bass. In : A Biological Survey of the Raquette Watershed. Suppl. 23rd Ann. Rept. N. Y. Cons. Dept. 1933 : 255–261.

——— and HUNTER, WANDA SANBORN. 1929 Studies on the parasites of fishes of the Lake Champlain Watershed. In : A Biological Survey of the Lake Champlain Watershed, Suppl : 19th Ann. Rept. N. Y. Cons. Dept. 241–260.

——— and ——— 1930 Studies on fish parasites in the St. Lawrence watershed. In : A Biological Survey of the St. Lawrence Watershed. Suppl. 20th. Ann. Rept. N. Y. Cons. Dept. (5) : 197–216.

——— and ——— 1934 Further studies on fish and bird parasites. In : A Biological Survey of the Mohawk-Hudson Watershed. Suppl. 24th Ann. Rept. N. Y. Cons. Dept. (9) : 267–283.

——— and ——— 1938 Studies on host reactions to larval parasites. I. The effect on weight. J. Parasit. **24**: 477–481.

——— and ——— 1942 Studies on host-parasite reactions. V. The integumentary type of Strigeid cyst. Trans. Amer. Microsc. Soc. **61**: 134–140.

LA RUE, G. R., BUTLER, E. P. and BERKOUT, P. G. 1926 The eye of fishes, an important habitat for larval Strigeidae. Trans. Amer. Microsc. Soc. **45**: 265–288.

MACKENTHUN, KENNETH M. 1946 A preliminary report on the age, growth and condition factor of Southern Wisconsin fishes. Wis. Cons. Dept. Invest. Rept. 574. (unpublished report)

NIGRELLI, ROSS F., and JAMES W. ATZ. 1943 Biometry of puffers and their parasites. Zoologica (New York). **28**: 1–8.

PALMER, EDDY S. 1939 Diplostomiasis, a hatchery disease of fresh-water fishes new to North America. Prog. Fish Culturist. (45) : 41–47.

SHETTER, DAVID S. 1936 Shrinkage of trout at death and on preservation. Copeia. 1936 : 60–61.

VAN CLEAVE, H. J., and MUELLER, J. F. 1934 Parasites of Oneida Lake Fishes. Roosevelt Wild Life Anns. **3**: 161–334.

WOODBURY, LOWELL A. 1940 A quantitative study of parasites of fishes with special reference to *Clinostomum marginatum* in the perch of Walsh Lake, Michigan. Ph.D. thesis, Univ. of Mich., Ann Arbor, Mich. (unpublished)

THE OVER-WINTERING OF BLACK GRUBS AND YELLOW GRUBS IN FISH[1]

JACOB H. FISCHTHAL[2]

It had been stated by many Wisconsin anglers that although many of the panfish in some lakes were grubby when caught during the summer and fall seasons, they apparently were "free" from grubs when taken through the ice in the winter. The question naturally arises, "Do the grubs drop out of these fish in the winter?".

In order to obtain some basic data in answer to this query an experiment involving 30 fish was set up on October 16, 1944. The fish were tagged and the tag number and locations of all encysted black grubs (*Neascus spp.* metacercariae) and yellow grubs (*Clinostomum marginatum* metacercariae) were recorded for each on separate outline drawings of a perch or centrarchid. The fish were maintained in a raceway at the Spooner, Wisconsin fish hatchery and supplied with water from the Yellow River flowage. On April 24, 1945, a period of six months after the start of the experiment, the fish were examined for missing grubs.

Only 14 of the 30 fish harbored black grubs. They were three perch (*Perca flavescens*), one rock bass (*Ambloplites r. rupestris*), six pumpkinseeds (*Lepomis gibbosus*), and four bluegills (*Lepomis m. macrochirus*). The 14 fish had a total of 200 black grubs at the beginning of the experiment. In the ensuing six months of over-wintering, only six or three per cent of these were lost, a rather small amount. Of the three perch, infected with 5, 7 and 12 grubs, respectively, the one with 7 had lost three parasites. This is a 12.5 per cent loss from the total of 24 grubs originally present on all perch. The rock bass with 23 grubs had lost two or 8.8 per cent. The six pumpkinseeds, with 2, 4, 11, 15, 33 and 56 grubs, respectively, did not lose any of their infection. Of the four bluegills, with 5, 6, 7 and 14 grubs, respectively, the latter fish had one cyst showing signs of coming out. Partially protruding from the center of the black-pigmented spot was a clear, thick, lens-like cyst with the grub inside. Of the total of 32 grubs present on all bluegills, only three per cent was lost.

All 30 fish were infected with yellow grubs. They were 19 perch, one rock bass, six pumpkinseeds, and four bluegills. These fish had a total of 324 grubs at the start of the experiment. In the course of the six months over-wintering, only 14 or 4.3 per cent of the grubs were lost, again a rather small amount. Eight or 42.1 per cent of the 19 perch lost some yellow grubs, however, the amounts were negligible when considering the numbers originally present on these fish. They harbored a total of 301 grubs at the start and only 11 or 3.98 per cent were lost. The perch were infected with from one to 39 grubs, averaging 15.8 per fish. The one rock bass lost its one grub. Of the six pumpkinseeds, four with one grub each, one with five, and one with nine, respectively, the latter fish had lost one grub. This is a 5.5 per cent loss from the total of 18 grubs originally present on all pumpkinseeds. The four bluegills each had one grub, but only one lost its infection.

CONCLUSION

From the foregoing data it is quite apparent that the over-wintering loss of grubs in panfish is negligible. The data certainly does not warrant the assertion that the fish are "free" from grubs when taken through the ice in the winter as many Wisconsin anglers had remarked.   It is the author's opinion that the fewer grubby fish taken in the winter can best be explained by the differences in the summer and winter fishing habits of the angler and the nature of the standing panfish population. During the summer the angler fishes for panfish inshore, for the most part in the shallow, weedy portions of the lake where infected snails and the panfish are frequently present in a close association.   This would tend toward grubby fish. However, there exists at the same time a portion of the panfish population which spends little time in the weedy areas where they may be exposed to infection, but tends to dwell in the non-weedy inshore regions and the inhabitable deeper, open water portions of the lake.   These fish would be almost entirely, if not entirely, free of grubs, since the snail population generally is inshore among the weeds.   With the advent of winter the inshore panfish population moves into deeper water. During the winter it is the habit of the angler to usually fish in the deeper, offshore waters of the lake and, therefore, he would catch more of the grub-free panfish population. This would lead to the exaggerated belief that the panfish were "free" of grubs when taken through the ice in the winter.

SOME METHODS FOR THE QUANTITATIVE STUDY OF ENTOZOIC AMOEBAE IN CULTURES*

A. M. Griffin and W. G. McCarten

School of Medicine, The George Washington University, Washington 5, D. C.

Quantitative studies of entozoic amoebae in cultures are beset with a number of difficulties. The haemocytometer method, as originally suggested by Paulson (1932), is statistically unsatisfactory unless the number is in excess of 100,000 per 10 ml. of culture; and such devices as counting the entire number in a cover-slip preparation of known volume are too time consuming for large-scale comparative studies. Trophic amoebae are rather sensitive to manipulation, hence centrifugation and resuspension in a reduced volume result in a considerable and by no means consistent reduction in numbers. Furthermore the excess rice starch necessary for maximum growth, together with other debris, forms so dense a background that accurate counts are quite impossible.

We have, therefore, attempted to develop techniques which make it possible to estimate low density populations and improve the ease and rapidity with which this can be done.

MATERIALS AND METHODS

1. *The Counting Chamber.* All counts are made in a chamber designed for counting the cells in spinal fluid. It is 0.2 mm. deep and bears the Fuchs-Rosenthal ruling. The sample available for counting is therefore about 3.7 times that contained in an haemocytometer.

2. *Rice Starch* is prepared by a modification of the method used by Snyder and Meleney (1943).

    1. To 100 gm. of rice powder (Difco) add sufficient distilled water to give a thick slurry. Add 5 ml. of chloroform and grind in a ball mill for 6–8 hrs.

    2. Transfer to 1000 ml. graduate, add distilled water to 1000 ml. Mix by inverting several times.

    3. Allow to sediment 20 min. or until a sharp line of demarcation appears, whichever is longer, and siphon off the supernate.

    4. Repeat until few starch grains remain in the sediment. Discard the sediment.

    5. Collect the starch from the combined supernates by centrifugation and resuspend in an equal volume of distilled water.

    6. Add 0.5 gm. Difco Trypsin 1 : 250 and adjust to pH 8.0 with 10 percent sodium carbonate. Add 5–10 ml. of toluene.

    7. Incubate at 37° C., readjusting the pH to 8.0 until it remains constant for 4 hrs. or longer.

    8. Centrifuge. Discard supernate and brownish upper layer of sediment.

    9. Resuspend in distilled water and repeat until no more of the brownish material appears on the surface of the sediment.

10. Resuspend in distilled water.   Add 1/3 volume of toluene and shake vigorously.
11. Centrifuge.   Remove with a pipette any material lodged in the water-toluene interface.
12. Repeat (10) and (11) until the interface is practically clear.
13. Discard the supernate.   Suspend in an equal volume of acetone.   Centrifuge and discard the supernate.   Repeat twice.
14. Remove the acetone from the packed starch *in vacuo.*
15. Dry to constant weight at 120° C.

Starch so prepared is an impalpable powder, suspends uniformly, consists of individual grains and contains no microscopically detectable foreign matter.   It is sterilized in weighed amounts for 2.5 hrs. at 150° C.

3. *The Medium* is a fluid medium based on Cleveland and Collier's (1930) and Chang's (1942) mediums.

A. *Liver Infusion.*
1. Infuse 100 gm. of Bacto Liver in 500 ml. of distilled water with continuous stirring for 1 hr. at 50° C.
2. Without stirring raise to 80° C. and hold for 10 min.
3. Filter by suction while hot and add distilled water to 2000 ml.
4. Add:

Bacto proteose-peptone	10.0 gm.
$Na_2HPO_4 \cdot 12H_2O$	23.9 gm.
NaCl	10.0 gm.

The pH should be 7.2.   If not, adjust with NaOH or HCl as indicated.
5. Autoclave for 15 min. at 15 lbs.
6. Add approximately 20 gms. of "Celite" Analytical Aid, mix and filter by suction.
7. Autoclave for 15 min. at 15 lbs. in capped flasks and store under refrigeration until needed.

B   *Buffered Salt Solution.*

NaCl	4.0 gms.
$Na_2HPO_4 \cdot 12H_2O$	7.8 gms.
KH_2PO_4	1.6 gms.
Distilled water to	1000.0 ml.

This gives an M/30 phosphate buffer in 0.4 percent NaCl.   The quantities of phosphate given are for a particular batch and should be so determined as to give a pH of 7.4.

Mix 1 volume of (A) with 2 volumes of (B) and add 10 percent of beef serum. Sterilize by filtration and dispense in 10 ml. amounts.

Suspend sterile rice starch in sterile medium—a 5 percent suspension is most convenient—and transfer aseptically the required amount to each tube of medium. Incubate at least 24 hrs. before use to test for sterility.

EXPERIMENTS

The type of investigation carried out in this laboratory uses the complete factorial design for all experiments.   Since a number of repeated determinations must be made at a single stage it has been our custom to fix the cultures by adding 1.0

ml. of formalin so that they may be counted at leisure. This procedure was satisfactory for comparative studies where the yield per culture was in excess of 100,000 so that counts could be made on the unconcentrated cultures. With lower yields, where concentration was required, enumeration was difficult if not impossible since excess starch and debris were concentrated along with the amoebae.

Physical purification of the starch as described above was only partly effective, and attempts to adjust the amount of starch to a point where the amoebae would consume most of it resulted in gross irregularity of yield. We found that a considerable excess had to be added since the amoebae tend to colonize and take up only those starch grains immediately adjacent to the colony. We therefore attempted to solubilize the starch after fixation of the amoebae.

Forty-five tubes of L-S-B (Liver-Serum-Broth) medium containing 25 mg. of purified rice starch were inoculated with 0.1 ml. of a 3 day culture of an *Endamoeba*

TABLE 1.—*Yield per Culture after Treatment. Yield* $\times 10^{-4}$

Min. at 70° C.	N–HCl ml.	Formalin, ml.			
		0.0	0.5	1.0	Mean
0	0.0	194	146	148	162.7
	1.0	132	158	128	139.3
	Mean	163.0	152.0	138.0	151.0
2	0.0	153	120	163	145.3
	1.0	159	121	139	139.7
	Mean	156.0	120.5	151.0	142.5
4	0.0	159	119	129	135.7
	1.0	180	165	163*	169.3
	Mean	169.5	142.0	146.0	152.5
8	0.0	178	157	128*	154.3
	1.0	184	174	109*	152.3
	Mean	176.0	165.5	118.5	153.3
16	0.0	222	166*	162*	183.3
	1.0	174	154*	162*	163.3
	Mean	198.0	160.0	162.0	173.3
Mean	0.0	181.2	141.6	146.0	156.3
	1.0	163.8	154.0	140.0	152.8
	Mean	172.5	148.0	143.1	154.5

* Starch eliminated.

sp. isolated in this laboratory. This organism is similar to *E. terrapinae* described by Sanders and Cleveland (1930). The inoculum contained 200,000 amoebae with virtually no cysts. The cultures were incubated 3 days at 30° C. On the third day 20 mg. of starch were added to each tube and the cultures further incubated for 1 hr. to permit the amoebae to gorge themselves with starch grains.

The cultures were then divided into 5 blocks of 9 each. Additions were made within the blocks as indicated in Table 1 except that 3 tubes in each block received 0.5 ml. of N–HCl. These are omitted from the table as a dense precipitate occurred which entirely prevented counting. The cultures were then heated at 70° C. for the times indicated.

The data of Table 1 are analyzed in Table 2. The preliminary analysis shows only one highly significant source of variability which is associated with formalin treatment. Heat treatment and the Heat × HCl interaction (A × B) had barely significant mean squares. However, when the non-significant sums of squares are

TABLE 2.—*Analysis of Variance of Treatments*

Source of Variation	df	Mean Square
Main Effects.		
(A) Heat Treatment.	4	744*
(B) HCl "	1	90
(C) Form. "	2	2481**
Interactions.		
A × B	4	770*
A × C	8	450
B × C	2	580
A × B × C	8	198

* Significant P = 0.01 to 0.05.
** Highly significant, P = less than 0.01.

pooled the only remaining significant mean square is that due to formalin treatment, (m.s. = 2481, d.f. = 2; error m.s. = 467, d.f. = 27).

We were rather surprised to observe that the starch was eliminated only in those cultures which were subjected to heat and formalin treatment with or without HCl. Heat alone or combined with HCl was ineffective although the starch grains were swollen and distorted. The optimum time of heating was between 8 and 16 min., although longer heating did not impair the appearance of the amoebae or their num-

TABLE 3.—*The Effect of the Sequence of Treatment.   Yield × 10^{-4}*

Replicate No.	Treatment				
	None	Heat	Form.	H–F	F–H
1	265	204	191	221	259
2	292	234	207	205	206
3	268	197	218	178	179
4	251	176	201	167	199
5	245	240	192	224	180
6	192	190	192	225	146
7	228	171	214	171	182
8	291	190	206	214	147
9	185	222	185	283	182
10	247	211	163	277	223
Mean	246.4	203.5	196.9	216.5	190.3

bers. The temperature is not critical provided that it be above 65° C. Even boiling does no harm although the contrast between amoebae and background is somewhat reduced.

Since the data of Table 1 indicated that the variability was primarily between those cultures receiving no treatment and the treated group, an experiment was designed to resolve the variation. Fifty tubes were inoculated as before and 10 cultures were subjected to each of four treatments: (1) heat at 70° C. for 10 min., (2) addition of 10 percent formalin, (3) heat followed by formalin, and (4) formalin followed, after 1 hr., by heat. The remaining cultures received no treatment and were counted immediately.

The results are given in Table 3 and the analysis of variance in Table 4 (A).

TABLE 4.—*Analysis of Variance of Sequence of Treatments*

Source of Variation	df	Mean Square
(A) Treatments	4	4917**
Error	45	1019
(B) Treatments	4	1676
Error	45	786

** Highly significant, P = less than 0.01.

The variability between treatments is now highly significant as compared with that among cultures treated alike.   It belatedly occurred to us that the untreated cultures were counted immediately and therefore showed minimal effects of manipulation. We therefore recounted them about 4 hrs. later and found a mean count of 182.6 as contrasted with the original mean count of 246.4.   This was a highly significant reduction and when the redetermined values are substituted the analysis of variance as given in Table 4 (B) shows no significant variability.

As was anticipated, the starch was removed only by treatment with formalin followed by heat.   In spite of the fact that HCl treatment did not render the starch soluble under the conditions of the experiment, we felt it desirable to rule out acidity of the formalin as the primary agent.   The particular lot used in these experiments had a pH of 3.3.   When adjusted to pH 7.0 or to pH 8.9 the action was still the same.   Furthermore neither formic acid at pH 3.3 nor sodium formate at pH 7.0 and pH 8.9 was effective in eliminating the starch.

These findings have led to the establishment of a routine for counting cultures of amoebae which has since been used with several hundred cultures of the *Endamoeba sp.* mentioned above, as well as with several sub-strains of the NRS strain of *E. histolytica*.   The result has been uniformly satisfactory.   The routine is as follows:

1. Suspend the sediment by "snapping" the tube or by gentle aspiration with a pipette.
2. Add 1.0 ml. of formalin and mix.
3. Suspend homogeneously and heat for 10 min. at 70° C.
4. Suspend and count.
5. If the count is less than 30 per sq. mm. centrifuge 5 min. at 2500 rpm (clinical centrifuge), and resuspend in a volume calculated to give a count between 30 and 100 per sq. mm.

DISCUSSION

The methods advocated in this report facilitate enumeration of entozoic amoebae in cultures and make possible concentration of cultures to a point where crude estimates may be made of yields of the order of 300 and statistically satisfactory counts may be obtained with yields of 3000.   Accuracy is further increased by the clarity with which the amoebae stand out against the background.

The chief disadvantage is that the necessary manipulations result in the loss of some of the organisms, probably by mechanical disintegration of senescent cells. However, the results obtained are more constant and, for comparative studies, more satisfactory.   This is especially true where replications are required during the time needed to make the counts, the changes in numbers in living cultures are greater than the statistical tolerance.   As we indicated above, the factorial design of our experiments often requires the manipulation of several hundred tubes within a relatively short time.   It would be physically impossible to make replicate counts on living cultures, but the treated cultures may be stored and counted over a period of weeks.

We do not attempt to explain the effect of formalin on starch grains.   The starch is not dissolved but is gelated in much the same way as when subjected to boiling.   On centrifugation swollen transparent "ghosts" of the original starch

grains are recovered against which the amoebae stand out in sharp relief.   We have been satisfied to demonstrate that the pH of the formalin is not the controlling factor.

SUMMARY

1. Undesirable debris can be eliminated from cultures of entozoic amoebae by using purified starch.

2. Residual starch may be removed by treating the cultures with 10 percent of formalin followed by heating to 70° C. for 10 minutes.

3. Manipulation results in a decrease in the absolute count but the relative counts remain constant.

4. The effect of formalin on rice starch is not associated with the pH of the system.

REFERENCES

CHANG, S. L. 1942 Studies on *Entamoeba histolytica*. I. Effect of hydrogen-ion concentration on encystation of *E. histolytica* in culture. Amer. J. Trop. Med. **22:** 471–492.

CLEVELAND, L. R. AND COLLIER, J. 1930 Various improvements in the cultivation of *Entamoeba histolytica*. Amer. J. Hyg. **12:** 606–613.

PAULSON, M. 1932 An accurate method for the numerical determination of *Entamoeba histolytica* in vitro and its possible use with other intestinal protozoa. Amer. J. Trop. Med. **12:** 387–400.

SANDERS, E. P. AND CLEVELAND, L. R. 1930 The morphology and life cycle of *Entamoeba terrapinae,* spec. nov., from the terrapin, *Chrysemys elegans*. Arch. f. Protist. **70:** 267–272.

SNYDER, T. L. AND MELENEY, H. E. 1943 Anaerobiosis and cholesterol as growth requirements of *Endamoeba histolytica*. J. Parasit. **29:** 278–284.

OCCURRENCE OF *PHLEBOTOMUS (NEOPHLEBOTOMUS)*
SHANNONI DYAR IN FLORIDA (DIPTERA,
PSYCHODIDAE)[1]

D. C. Thurman, Jr.,[2] J. A. Mulrennan[3] and Ernestine B. Thurman[4]

The first report of the occurrence of blood sucking Psychodidae in the United States was made by Coquillet (1907). Dampf (1938) mentioned collecting specimens of *Phlebotomus* from Florida but did not name the species collected; Johannsen (1943) reported that a species of *Phlebotomus* was collected from the Okefenokee swamp in 1912 while biting. Rozeboom (1944) reported *Phlebotomus limai* Fonseca (a synonym of *P. shannoni* Dyar, 1929)[5] from Alabama, Mississippi and North Carolina with some of the females taken while biting.

Four small biting flies, which represent the first specific record of a species of *Phlebotomus* found in Florida, were taken at Palm Valley, St. Johns County, April 5, 1948 by Dr. M. W. Provost, Miss Lucile Logan and two of the authors, and were determined to be *Phlebotomus*. Later the specimens were identified by Dr. G. B. Fairchild of the Gorgas Memorial Laboratory, Panama, as *Phlebotomus shannoni* Dyar, 1929.

In the literature six species of *Phlebotomus* are known from the United States. Rozeboom (1944) listed four species other than *P. shannoni:* "*P. Vexator* Coquillet, which is widespread in the southern states having been taken from Maryland to California; *P. diabolicus* Hall, from Uvalde, Texas; *P. texanus* Dampf. from San Antonia, Texas; and *P. stewarti* to be described by Mangabeira and Galindo, from California." (The description of *P. stewarti* appeared as *P. stewarti* Mangabeira and Galindo, 1944). Another species, *Phlebotomus (Dampfomyia) anthophorus* Addis, was recorded by Addis (1945) from Uvalde, Texas. Addis has also prepared an excellent key to the males and females of the United States species (see Addis, 1945a).

P. shannoni was originally described by Dyar (1929) from three males collected by R. C. Shannon at Cano Saddle, Gatun Lake, Panama, May, 1923. The record of this species in Florida extends the known range of the species in the United States to include almost all of the southeastern states.

The *Phlebotomus* sp. believed to occur in the Okefenokee swamp in Georgia may well be *P. shannoni* which is a man-biting species in Florida and other surrounding states. The females of *P. shannoni* reported in this paper were biting when captured at 8:30 P.M., on a cloudy, dark night at the time a collection of biting mosquitoes was being made. The females attacked below the waistline, seeking exposed portions of the legs and ankles.

The Palm Valley area, from which the collection of *P. shannoni* was made, is a semi-swamp with numerous pines (slash-pine, *Pinus palustris*) and cabbage-palms (*Sabal palmetto*) forming a forest ceiling which partially shades the ground. The area merges from wet flatwoods into wet hammock with an intersprinkling of magnolia (*Magnolia grandiflora*), pignut (*Hicoria giabra*), red-bay (*Tamaia borbonia*), holly (*ilex opaca*), and other types of vegetation which presage the typical development of a Florida magnolia climax forest. On the forest floor, which is thinly sprinkled with vines and creepers, there is a rich mat of organic material formed by the decaying of leaves, logs, vines and other debris. During the wet season the major portion of the forest floor in this area may be covered with water for several days at a time, while even during the dry season the shaded ground is moist. The collecting area is located about one-half mile west of the Florida intercoastal waterway which contains brackish water subject to tidal action.

The wide variety of aquatic and semi-aquatic habitats present in the area provides an ideal situation for the development of a large number of species of insects, especially important pest mosquitoes. Though two subsequent collecting trips were made, larvae of *P. shannoni* were not found; however, it is felt that were the opportunity afforded for a more thorough study, a great deal could be learned about this interesting insect.

REFERENCES

ADDIS, C. J. 1945 *Phlebotomus (Dampfomyia) anthophorus,* n. sp. and *Phlebotomus diabolicus* Hall from Texas. (Diptera; Psychodidae). J. Parasit. **31:** 119–127.

ADDIS, C. J. 1945a Collection and preservation of sandflies (*Phlebotomus*) with keys to the U. S. species. (Diptera; Psychodidae). Trans. Amer. Micro. Soc. **54:** 328–332.

BARRETTO, M. P. 1946 Sobre a sinonimia de *Flebotomos* americanos. An. Fac. Med. Univ. S. Paulo. **22:** 1–27.

COQUILLET, D. W. 1907 Discovery of blood sucking Psychodidae in America. Ent. News. **18:** 102.

DAMPF, A. 1938 Un nuevo *Phlebotomus* (Insecta, Diptera, Fam. Psychodidae) procedente de Texas, E. U. A. Anal. Esc. Nac. Cien. Biol. **1:** 119–122.

DYAR, H. G. 1929 The present knowledge of the American species of *Phlebotomus* Rondani. (Diptera, Psychodidae). Amer. J. Hyg. **10:** 112–124.

JOHANNSEN, O. A. 1943 A noxious species of *Phlebotomus* in the Okefenokee swamp in Georgia. Psyche, **50:** 112–113.

MANGABEIRA, F. O. and GALINDO, P. 1944 The genus *Flebotomus* in California. Amer. J. Hyg. **40:** 182–198.

ROZEBOOM, L. E. 1944 *Phlebotomus limai* Fonseca in the United States. (Diptera, Psychodidae), J. Parasit. **30:** 274–275.

TROMBICULID MITES AFFECTING MAN. III. *TROMBICULA (EUTROMBICULA) SPLENDENS* EWING IN NORTH AMERICA

DALE W. JENKINS

Medical Division, Army Chemical Center, Maryland

In nearctic North America two species of chigger larvae are known to be important widespread pests that cause human trombidiosis. These are *Trombicula (Eutrombicula) alfreddugèsi* (Oudemans) and *Trombicula (Eutrombicula) splendens* Ewing. In the tropical and subtropical areas of the United States *Trombicula (Eutrombicula) batatas* (L) and *Trombicula (Eutrombicula) alfreddugèsi* subsp. *tropica* (Ewing) are also of local importance in attacking human beings. *T. alfreddugèsi* is known in both the adult and larval stages, but *T. splendens* is known only from 6 adults from Wisconsin. *Acariscus masoni* Ewing was described on the basis of larvae from Florida, and the present author considers these larvae to be the same species as *T. splendens*. The correct synonymy of this species appears to be the following:

Trombicula (Eutrombicula) splendens Ewing

Trombicula splendens Ewing, 1913, Bull. Amer. Mus. Nat. Hist. 32(5): 113–114. Ewing, 1920, Ann. Ent. Soc. Amer. 13: 381. Ewing, 1926, Ent. News. 37: 111–113. Vitzthum, 1929, Zeit. f. Parasit. 2(2): 234. van Thiel, 1930, Parasitology, 22: 353. (refers to *T. splendens* Howard, 1918!). André, 1930, Mem. Soc. Zool. France. 29: 109. Ewing, 1931, Proc. U. S. Nat. Mus. 80: 1–19. Ewing, 1933, Proc. U. S. Nat. Mus. 82: 1–6. Ewing, 1937, Proc. Biol. Soc. Wash. 50: 169. Sig Thor and Willman, 1947, Das Tierreich. Lfg. 71b: 344.

Gen.? sp.? "Irritating chigger" Howard, 1918, Seventeenth Rep. State Entom. Minn. 117: 111–144. (figure of *T. splendens* larva)   (New synonymy)

"American chigger" Ewing, 1921, (Not of Riley, 1873) U. S. Dept. Agr. Bull. 986: 1–19. (figure of *T. splendens* larva)   (New synonymy)

Acariscus masoni Ewing, 1943, Proc. Ent. Soc. Wash. 45: 60. Farner, 1946, Proc. Ent. Soc. Wash. 48(2): 32. Farner and Seaman, 1946, J. Parasit. 32: 93. Farner, 1947, Proc. Biol. Soc. Wash. 60: 29–30. Jenkins, 1946, CWS Quarterly Prog. Rep. 10: 34–38. (New synonymy)

Eutrombicula masoni (Ewing) Jenkins, 1947, Ann. Ent. Soc. Amer. 40: 56–68. Jenkins, 1948a, Amer. J. Hyg. 48: 22–35. Jenkins, 1948b, Amer. J. Hyg. 48: 36–44. (New synonymy)

This species was first discovered in the adult stage in 1909 under stones in a tamarack bog in Wisconsin and was named *Trombicula splendens* by Ewing in 1913. No other adult specimens of this species have been reported since that time. The larvae were discovered in Minnesota and figures were published by Howard in 1918 and by Ewing in 1921, but they were neither identified nor correlated with the adults. The larvae were finally described from Florida specimens and named *Acariscus masoni* by Ewing (1943), but the adults of this latter species have remained undescribed.

The present author reared chigger larvae collected in the type locality of *A. masoni* to adults and obtained second generation larvae using the methods of Jenkins (1947). These larvae were compared with the type larval specimens of *A. masoni* and were found to be the same (fig. 1). The large number of reared adults which produced *A. masoni* larvae were compared with all existing type specimens of *T. splendens* and they were found to be indistinguishable. The type series of *T.*

---

splendens consists of a type adult in poor condition in the U. S. National Museum, and a cotype adult in the American Museum of Natural History, which was kindly loaned by Dr. C. D. Michener. These types were remounted in polyvinyl alcohol and the cotype is illustrated in figures 2 and 4. Thus *Trombicula* (*Eutrombicula*) *splendens* Ewing appears to be the adult of *Acariscus masoni* Ewing larvae, and *A. masoni* becomes a synonym. Reared adults and correlated larvae of *T. splendens* were deposited by the author in the U. S. National Museum.

In the original description of the adult by Ewing (1913) the eyes are said to be absent, and in (1920) the eyes were observed to be indistinct, and it is stated that the sensillae (pseudostigmatic organs) originate between the sensillary bases (pseudo-stigmata). In the type, cotype, and reared adults, the eyes are readily observed, and the sensillae originate from the centers of the sensillary bases as shown in figure 2. The larvae of *T. splendens* appear to be very closely related to *Trombicula* (*Eutrombicula*) *wichmanni* (Oudemans) of the Southwest Pacific area. The adults and larvae can be distinguished from the closely related *T. alfreddugèsi* by the characters shown in table 1. In addition to the morphological characters, the habitat, distribu-

TABLE 1.—*Distinguishing characters of larvae and adults of* T. splendens *and* T. alfreddugèsi

	T. splendens	*T. alfreddugèsi*
Larvae		
No. of dorsal setae	(26) arranged 2–6–6–4–4–2–2	(22) arranged 2–6–6–4–2–2
No. of ventral setae	(16) arranged 2–2–6–2–2–2	(14) arranged 2–2–6–2–2
Ave. length dorsal setae (longest)	49 µ	42 µ
Shape of scutum	corners rounded, not thickened around bases of marginal setae, posterior margin often more convex	corners less rounded, thickened around bases of marginal setae, posterior margin less convex
Adults		
No. of apical nude setae on palpal tarsus	9–11	7–8
Width between sensillae	55–70 µ	48–51 µ

tion, and physiological requirements of the two species are different. The standard measurements and more detailed descriptions of the stages of these species will appear in a forthcoming revision of the subgenus (*Eutrombicula*) in relation to trombidiosis in the American hemisphere. The larvae of *T. splendens* are somewhat variable, and a posterior pair of dorsal setae may be absent, leaving 24 dorsal setae. In the southern United States it is not uncommon to find white or slightly pinkish larvae instead of the usual red ones. This variation occurs in local populations and is apparently a genetic albino character, although no morphological structures have been found to be correlated with it. White larvae have also been obtained in rearing colonies.

The larvae of *T. splendens* are the most important cause of human trombidiosis in the southeastern part of the United States especially in Florida. They are most abundant in swamps, bogs, and moist places near coastal areas and inland about 150 miles from Texas to Florida and north to Massachusetts. This species is also found through the Great Lakes Region and up the Mississippi River valley to Minnesota. A map of the distribution of this species by counties was presented by Jenkins (1948a). The known distribution includes only nearctic North America, and specimens have been identified by the author from the following states: Minnesota, Wisconsin, Michigan, Massachusetts, Delaware, Maryland, District of Co-

lumbia, Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Texas, Arkansas, and Tennessee. It has been reported from Kansas and Nebraska. This species was also identified by the author from Welland Co., Ontario, Canada, (specimen in the collection of the Rocky Mountain Laboratory, Hamilton, Montana).

Larvae of *T. splendens* have been used in extensive tests in relation to chigger repellents, acaricide impregnation of clothing, and chemical area control of the larvae. The bionomics and behavior of this species are discussed by Jenkins (1948a & b) and 42 species of vertebrate host animals are known, including mammals, birds, reptiles, and amphibians. Reptiles, especially snakes and turtles, appear to be the most important natural hosts. Human beings are readily attacked by the larvae and a severe inflammatory reaction is produced.

EXPLANATION OF PLATE I.

FIG. 1. Larva of *Trombicula splendens* Ewing, dorsal view on right, ventral view on left. (legs rotated beyond coxae).

FIGS. 2–4. Adult cotype of *T. splendens*. 2. Scutum showing eyes posterolateral to sensillary bases, sensillae, saddle between sensillary bases, carina and crista. 3. Inner view of right palp showing palpal claw, tarsus, 3 ctenidia and a spine. 4.Posterior body hair. (Drawings by Miss Alma D. Dinehart, U.S.P.H.S., Rocky Mountain Laboratory, Hamilton, Montana, and the author).

PLATE I
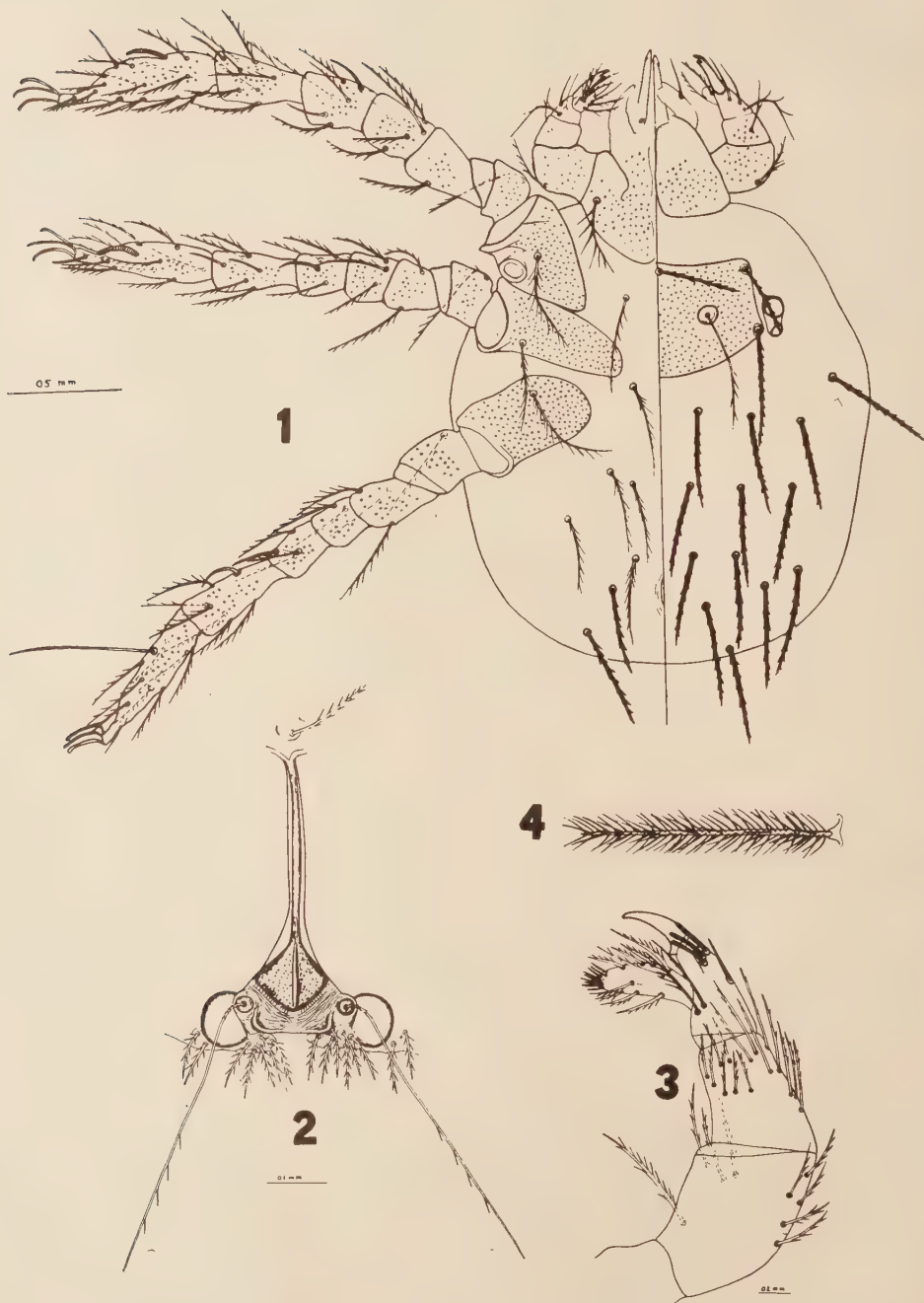
A THEORY OF EGG–DEPOSITION BY *BILHARZIA HAEMATOBIA*

Claude H. Barlow, M.D., ScD.*

INTRODUCTION

Various theories of egg-laying by *Bilharzia haematobia* (*Schistosoma haemato-bium*) and the evacuation of their eggs from the body have been advanced, which seem to me to come just short of being logical enough to be convincing, and to lack clinical evidence enough to make them tenable.

The following theory seems to me to meet these objections and to have clinical facts enough to uphold it.

THEORY

After the process of egg-laying, the progress of eggs from the worms to the bladder, the development of the eggs in the body and their final escape by means of the urine is at every stage a process of histolysis. It is even quite logical to assume that the female worm may find exit from the venules through a deposition of histo-lytic secretion at the point of exit and also that there may be a use of histolytic secretion in forming egg receptive cysts in the bladder wall.

SEQUENCE OF EVENTS

Let us suppose that the female worm, lying in the venule near the mucosa of the bladder, either by force or by histolysis, pushes out through the vein wall and down close to the inside of the bladder wall where she makes a small pocket or cyst. Into the cyst so formed she begins to lay eggs and continues until the cyst is completely filled. She then withdraws, leaving a small patent canal from the venule into the cyst.

The eggs in the cyst develop until the miracidia are mature. At maturity the miracidia exude histolytic substance which permeates the shell and enters the cyst. Its action may be presumed to be equal in all directions but there are two weak points in the encircling cyst wall; one where the worm entered for egg-laying, and one at the thin spot on the bladder wall nearest to the cyst wall. The thin site of the cyst wall and then the mucous membrane of the bladder-wall are histolyzed and the eggs escape into the bladder en masse during urination. When the bladder is empty the contraction of the bladder wall allows for clot formation at the site of rupture into the bladder and leaves the cyst in a state easily to be found by the female worm and refilled with eggs.

CLINICAL EVIDENCE

In other theories of egg-deposition and elimination it has been hypothesized that the eggs escape into the tissues and *find their way through* the tissues to the bladder. Let us suppose two worms laying eggs from two venules, one located twice the distance from the bladder as the other. One must accept that eggs would travel at relatively the same rate through the tissues. One egg would, therefore,

take twice as long as the other to arrive and would be more advanced in development than the other. This is not borne out by clinical examination as all eggs seen in routine examination are fully developed. Only one explanation of such a striking fact is tenable and that is that the eggs remain in the tissues until all of a batch are fully developed before being extruded into the bladder.

I have fed a patient excessive quantities of water over a 24 hour period, after which the urine discharged was filled with free-swimming miracidia. Even the few eggs which did not hatch were all fully mature.

Another clinical evidence of the theory was shown by examination of the sequential escape of unchecked urination.

Racks of 50 ml. centrifuge tubes were set ready and a heavily infected patient was allowed to urinate. The tubes were passed under the stream filling them up to the 50 ml. mark, in sequence. When the urination was completed the tubes were examined. This was repeated time after time for comparison.

From these examinations the following facts emerged:

1. The first part of the urine is clear and holds little freshly shed blood.

Explanation: the cysts had not yet ruptured.

2. The next part holds some blood and some casts. These casts deserve special attention. They are conical plugs of clot at the inner or cyst end of which a few eggs can be seen embedded in the clot, sometimes 2 or 3 and sometimes 7 or 8. Most of such eggs hold inactive miracidia within them. In length the casts are from 2 to 3 times as long as in diameter. They are blood fibrinous clots and contain no mucous cells. The casts appear as soon as the rupture occurs because they come out of the cysts before eggs can escape.

3. The next portion contains fresh blood in profusion and some eggs. After the extrusion of the plugs or casts there would be an escape of fresh blood but fewer eggs than later.

4. The last portion contains a heavy drenching of eggs, squeezed out by muscular contraction of the bladder wall.

Examination of my own urine while infected with Bilharziasis exhibited the same sequence of events.

In addition to this there is one bit more evidence which leads one to believe that the worms lie in close approximation to the site of egg escape. In my case there were small papules on the skin of the groin near the scrotal folds. Upon opening these papules they were found to be packed with eggs and a biopsy caught worms just under the skin, in favourable nearness for egg laying into the cysts. In experimental animals it is impossible to kill so quickly that one catches the worms *in situ*. Blood recedes from the arteries to the veins and worms seek escape. In the biopsy an attempt was made to catch the worms as nearly *in situ* as possible. In order not to disturb the worms, no anaesthetic was used and the cutting was done as rapidly as possible before the worms would retire. The attempt was successful. That the worms react quickly to such an assault was evidenced by a marked lessening of egg-discharge for a period of 10 days after which eggs appeared in large numbers in the urine, a medium of escape which they had not utilized largely before, the majority of eggs having used the rectal route.

As an evidence of histolytic action in the rupture of the cysts, I first became

aware of their presence by the bilharzial itch, an itch which is also typical of the invasion of cercariae and quickly recognized.

The uniform number of eggs appearing in daily urinations is also evidence against fortuitous progress through tissues to their exit into the bladder. The uniformity is even greater than it is in the case of ascariasis or ancylostomiasis where habitat is already in a viscus which has access directly to the outside of the body.

These deductions seem logical and they explain in an easily acceptable manner all of the steps in egg-deposition as seen in clinical examination. The one great hitch in the theory of fortuitously wandering eggs is the fact that all eggs present the same developmental maturity which would certainly not be the case if some took longer to find their way out than do others.

SULFAGUANIDINE THERAPY IN EXPERIMENTAL INTESTINAL COCCIDIOSIS (*EIMERIA ACERVULINA*) OF CHICKENS

MARION M. FARR

Zoological Division, Bureau of Animal Industry, Agricultural Research
Administration, U. S. Department of Agriculture

Tyzzer (1929) reported *Eimeria acervulina* to be the cause of a serious chronic type of coccidiosis of chickens, but later Tyzzer, Theiler, and Jones (1932) stated that they could not "demonstrate experimentally any pronounced pathogenicity" on the part of this species. Johnson (1931), on the other hand, presented evidence that severe infections of *E. acervulina* resulted in a temporary cessation in egg production by the affected birds. Dickinson (1939, 1941) and Dickinson and Scofield (1939) found that when massive doses of *E. acervulina* oocysts were administered to susceptible laying hens there was a drop in feed consumption, a loss in body weight, a change in the character of the feces, and an interruption in egg production. They also stated that as the birds began to recover from the disease they began to gain weight and resumed egg laying. Moreover, the last named authors reported that *E. acervulina* was the cause of the death of its bird host.

Levine (1941, 1943) reported that 0.5 percent sulfaguanidine mash, administered prior to inoculation of chickens with *E. acervulina* and fed thereafter continuously for the "duration of the experiment", prevented development of the parasite.

The purpose of the present investigation was to determine the effect of sulfaguanidine medication on *Eimeria acervulina*, when the drug was administered at different periods during the course of the infection.

PROCEDURE

The chickens used in the following experiments were either Rhode Island Reds or Barred Rock and Rhode Island Red crosses. The chicks were obtained as day-old birds from the Animal Husbandry Division of the Bureau of Animal Industry, Beltsville, Maryland. They were confined in battery brooders in an isolation room until the start of each test, at which time they were transferred to all-metal brooders or to wire cages. Containers fastened to the outside of the brooders and cages supplied feed and water to the birds. Papers were spread on the dropping pans underneath the wire floors of the brooders and cages, to facilitate the removal of the droppings. These papers were changed daily in order to prevent the ingestion of sporulated oocysts.

Just preceding each test, the birds were weighed and placed in groups of equal numbers and approximately equal weights. Throughout the tests the birds were weighed regularly—daily from the 4th to 10th day after inoculation, otherwise 2 to 4 times a week. At the end of each test, surviving birds were killed and carefully examined for lesions of coccidiosis.

For purposes of inoculation the oocysts were obtained from the small intestines of experimentally infected birds on the 5th day after inoculation. They were sporulated in 2.5 percent potassium dichromate solution and washed in distilled water.

TABLE 1.—*Effect of sulfaguanidine on Eimeria acervulina infections*

Experiment No.	Birds				No. oocysts administered	Treatment with sulfaguanidine mash	Test duration	Average weight gains	Necropsies Number of birds with lesions			
	Age	Breed	Number	Average weight					none	slight	moderate	severe
	(days)			(grams)			(days)	(grams)				
1	15	R.I.R.‡	25	111	325,000 in 4 daily doses	0.5% beginning 3 days after 1st dose and to end of test	12	97	25	..	..	..
			25	111	do	none (inoculated controls)		41	..	..	7	18 (1 died)
			25	111	none	none (uninoculated controls)		101	10 (rest not exam.)	..	..	..
2	20	R.I.R.	25	133	465,000 in 4 daily doses	0.5% beginning 5 days after 1st dose and to end of test	12	89	24	1†	..	..
			25	133	do	none (inoculated controls)		73	2	2	4	17
			25	133	none	none (uninoculated controls)		144	not exam.			
3	36	R.I.R.	11	312	494,000 in 1 dose at start of test. 305,000 in 1 dose on 29th day	1% beginning 5th day after 1st dose and for 5 days	39	621	6	2†	2	1
			11	312	305,000 in 1 dose on 29th day	none (inoculated controls)		616	9	2†	1	10
			11	312	do	none (resistance test controls)		533	..	..	..	..
4	19	R.I.R.	15	155	311,000 in 1 dose at start of test	0.5% beginning 4th day after dose and for 7 days	18	196	5*	2†	4†	4†
			15	157	do	none (inoculated controls)		155	8†	2†	2*	3*
			15	156	none	none (uninoculated controls)		214	15**	..	..	..
5	47	R.I.R.§ x B.R.	14	496	1,035,000 in 1 dose at start of test	0.5% for 4 days beginning at inoc. (T-O)	15	279	4	4	5	1
			14	500	do	0.5% for 4 days beginning 1 day after dose (T-1)		260	10	2	2	..
			14	498	do	0.5% for 4 days beginning 2 days after dose (T-2)		251	9	3	2	..
			14	489	do	0.5% for 4 days beginning 3 days after dose (T-3)		268	8	4	2	..
			14	505	do	0.5% for 4 days beginning 4 days after dose (T-4)		291	6	4	4	..
			14	501	do	none (inoculated controls)		221	12	2	..	..
			14	502	none	none (uninoculated controls)		369	14	..	..	..

* Examined at end of treatment period.
** 5 examined at end of treatment period, 10 examined at end of test.
† Examined at end of test.
‡ Rhode Island Red.
§ Rhode Island Red and Barred Rock cross.

Each bird was inoculated with a single dose or with 4 repeated doses, one on each of 4 successive days, by injecting the oocysts into the crop.   In experiment 3, all the birds, including those previously serving as uninoculated controls, received a large challenging dose of oocysts on the 28th day after the original inoculation.

Records of oocyst production, in thousands of oocysts per gram of droppings, were kept for each of the different groups.   The method of enumeration of oocysts in the droppings was the same as that described by Farr and Allen (1942).

The sulfaguanidine was mixed in the mash at a level of 1 percent by weight in experiment 3 and at a level of 0.5 percent in the other experiments.   Feed and water consumption records were kept in experiment 2 only.   Data on the tests are presented in table 1.

<div align="center">RESULTS</div>

Symptoms.—The symptoms exhibited by the inoculated controls consisted mainly of droopiness, ruffled feathers, loss of weight, and slimy droppings.   These symptoms appeared either at the end of the 4th day or on the 5th day after inoculation, and continued through the 7th day.   Following disappearance of these symptoms the chickens began to improve and, so far as external appearances were concerned, they had returned to normalcy within a week.   Only one bird (an inoculated control in experiment 1) died of the infection.

All the groups of birds which received the drug made better weight gains than the inoculated controls.   In experiment 1 the drug was fed continuously, beginning on the 3rd day after initial inoculation, and in this instance the weight gains of the treated birds approximated those of the uninoculated controls (table 1).   In experiment 5, when treatment was started at the time of inoculation and administered for the succeeding 4 days, the treated birds remained normal until the 8th day after initial inoculation (4 days after withdrawal of the drug).   Then they ceased eating, became droopy, and lost weight.   On the 10th day they began to recuperate and made good weight gains thereafter.   The only symptom shown by the birds which received treatment for 4 days, beginning 1, 2, and 3 days after inoculation, was a failure to make as good weight gains as the uninoculated controls.   When treatment was initiated on the 4th or 5th day after inoculation, the treated birds exhibited the same symptoms as the inoculated controls.   Figure 1 shows the weight records of the birds of experiment 3 in which treatment was started on the 5th day after inoculation.   As shown in the figure, the weight records of the treated birds and the inoculated controls were similar on the 5th and 6th days after inoculation, but on the 7th and 8th days the treated birds made better weight gains than the inoculated controls.   Thereafter the weight gains of the two groups more or less paralleled each other.   At the time of administration of the challenging dose the average weights of the treated group, inoculated controls, and resistance test controls were 837, 811, and 892 grams, respectively.

Figure 1 also presents graphically the effects of the challenging test dose on the chickens of experiment 3.   After this inoculation the treated birds and the inoculated controls made an average gain of 38 and 56 grams, respectively, while the resistance test controls lost an average of 105 grams.

During the last 9 days of experiment 2, the total average feed and water consumption per bird per group was as follows: Treated birds, 247 gms. feed, 606 cc.

water; inoculated controls, 251 gms. feed, 583 cc. water; uninoculated controls, 344 gms. feed, 875 cc. water. On the days when the treated birds and the inoculated controls lost weight, their intake of feed and water was about half the intake of the uninoculated controls.

Oocyst output.—The oocyst outputs of the groups of experiment 5 are shown in figure 2. In this experiment the drug was fed for 4 days only, and the most striking effect was a temporary interference with the oocyst discharge. Oocysts were absent from the dilutions prepared from the droppings of the T-O group (placed on treatment at the time of inoculation), and the T-1 and T-2 (placed on treatment 1 and 2 days, respectively, after inoculation) groups until the 8th, 9th,
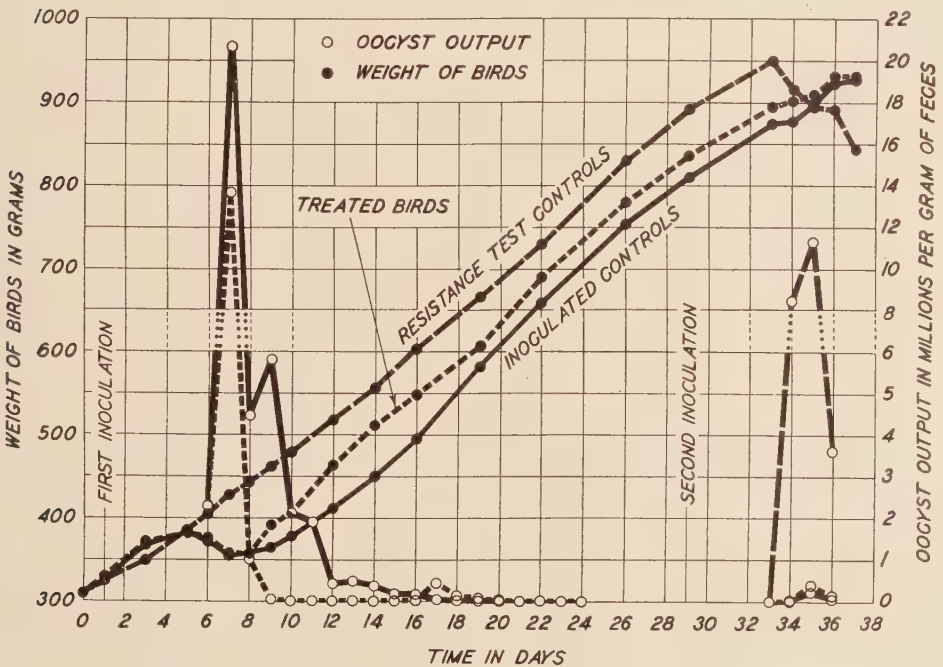

Fig. 1. The effect of 1 per cent sulfaguanidine mash on weight gains and on oocyst production. Chickens received 409,000 *E. acervulina* oocysts at 1st inoculation and a resistance test dose of 305,000 oocysts at 2nd inoculation. Sulfaguanidine administered from the 5th to the 10th day after 1st inoculation.

and 10th days, respectively, after inoculation, that is, in each case until 4 days after termination of therapy. The peak production of the T-O group exceeded that of the IC (inoculated controls) while the peak oocyst productions of the T-1 and T-2 groups were considerably below that of the IC. The oocyst output of the T-3 group (placed on treatment 3 days after inoculation) was markedly less than that of any other group, but there was no delay in the time of initial oocyst discharge. However, in this group the oocyst output dropped to a very low level 3 days after start of treatment and remained low until 5 days after termination of therapy when it rose somewhat.

When treatment was initiated on the 4th day after inoculation with a single dose of oocysts as in the T-4 group of experiment 5 and in the treated group of experi-

ment 4, the oocyst output on the 5th day after inoculation was very close to that of the inoculated controls. However, the oocyst outputs of these two treated groups dropped on the 6th day after inoculation and by the 7th day were at a very low level as compared with the inoculated controls. On the 5th day after the withdrawal of the drug the rate of oocyst discharge increased.

Figure 1 shows graphically the oocyst output of the birds placed on sulfaguanidine treatment on the 5th day after inoculation. As shown in the figure, the oocyst discharge of the treated and inoculated control groups rose very sharply on the day following institution of treatment; however, that of the latter reached a
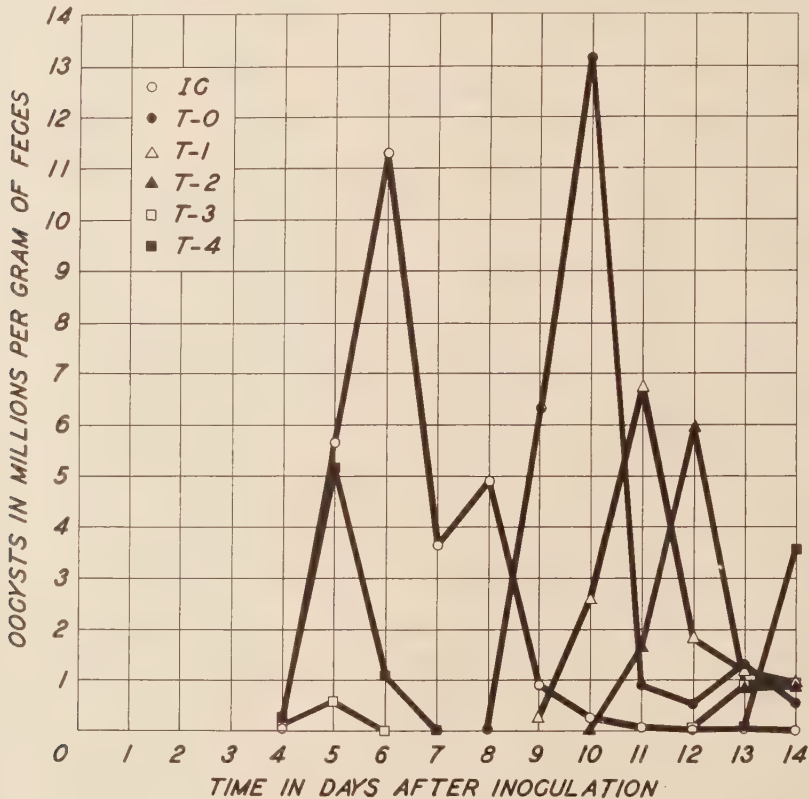

FIG. 2. The effect of a 4-day administration of 0.5 per cent sulfaguanidine mash on oocyst production. Chickens recived 1,035,000 *E. acervulina* oocysts in 1 dose. IC, inoculated controls; T-0, treatment started at time of inoculation; T-1, T-2, T-3, and T-4, treatment started 1, 2, 3, and 4 days after inoculation, respectively.

much higher level than that of the former. On the succeeding day, the oocyst output of both groups began to drop rapidly, that of the treated group continuing to drop during the next 3 days until on the 5th day of treatment it had declined to a level below that possible to detect by the method used. It remained far below the output of the inoculated controls until the 6th day after withdrawal of medication when it temporarily rose above the level of the inoculated controls. Following a rise on the 8th day after inoculation the oocyst output of the control group dropped very rapidly but, in general, it remained at a higher level than that of the treated birds.

Resistance to reinfection of the inoculated control and the inoculated treated groups is also shown in figure 1. Following reinoculation, the oocyst discharge of these 2 groups was very low as compared with the oocyst discharge of the resistance test control birds.

Necropsies.—As shown in table 1, the necropsies of treated birds at the time of withdrawal of sulfaguanidine showed that these birds were remarkably free of lesions. In experiments 1, 2 and 4, only one chicken of the 55 examined at the end of the period of treatment showed even a slight lesion of *E. acervulina* infection while 50 of the 54 inoculated controls examined at the same time had moderate to severe lesions. On the other hand, when the treated birds were examined 6 to 7 days after withdrawal of medication, as in experiment 4, most of them exhibited moderate to severe lesions while the remaining inoculated controls examined at the same time exhibited few if any lesions; the uninoculated controls showed no lesions. Table 1 shows this same condition in the treated groups of experiment 5 when they were examined 8 to 11 days after termination of therapy. The absence of lesions in the uninoculated controls indicates that the lesions found in the treated birds after they had been off the drug for 6 to 7 days were not due to extraneous infection.

The results of necropsies of birds at the end of experiment 3 showed that 9 of the 11 inoculated controls and 6 of the 11 treated birds had developed a strong resistance to reinfection. All of the 11 resistance test controls and 3 of the treated birds were susceptible to moderate or severe infection.

DISCUSSION

From observations on clinical symptoms, oocyst output, and necropsies, the benefit which the infected birds derived from the treatment seemed to bear some relation to the time which elapsed between inoculation and treatment. When treatment was initiated at inoculation and continued for 4 days, the effect was primarily a temporary prevention in loss of weight and delay in discharge of oocysts. When a 4-day treatment was initiated 1 or 2 days after inoculation, there was not only a delay in discharge of oocysts but also a reduction in oocyst production; also, there was no loss in weight. When treatment was initiated on the 3rd day after inoculation there was a marked reduction in oocyst output and there was no loss of weight. None of the birds on the 4-day treatment made as good weight gains as the uninoculated controls. Birds placed on treatment on the 4th or 5th day after inoculation made better weight gains than the inoculated controls and discharged fewer oocysts but exhibited the same symptoms as the inoculated controls. In the groups treated late in the infection, the greater weight gains of the treated birds seemed to be due to their more rapid recovery. In experiment 3, in which the birds were held for one month after initial inoculation, the inoculated controls had not reached the weight of the treated birds and the treated group in turn had not attained the weight of the uninoculated controls.

Necropsies of inoculated birds after 5 to 7 days of treatment revealed few, if any, macroscopic lesions, even in those groups placed on treatment as late as 4 days after inoculation. Therefore, the drug must have had some effect on every stage in the life cycle of the parasite.

Both the treated birds and the inoculated controls of experiment 3 had developed

a strong resistance to reinfection. It appears that the resistance of the treated group was not quite as effective as that of the inoculated controls, as evidenced by the fact that following reinoculation the treated birds passed more oocysts and had more lesions than the inoculated controls.

SUMMARY AND CONCLUSIONS

1. A series of tests was performed on 326 chickens to determine the efficacy of sulfaguanidine against *E. acervulina* infection.

2. The effect of a 4-day treatment with 0.5 per cent sulfaguanidine mash, beginning at the time of inoculation, was primarily coccidiostatic. Within 4 days after termination of therapy, the treated birds, which had been active and alert up to that time, became dull, lost weight, and passed large numbers of oocysts.

3. A 4–day treatment, beginning 1 or 2 days after inoculation, resulted in a 4-day delay in oocyst discharge and a moderate reduction in numbers of oocysts produced. These birds failed to make as good weight gains as the uninoculated controls.

4. Birds treated with sulfaguanidine mash beginning 3 days after initial inoculation of 4 daily doses of oocysts, and continuing treatment to the end of the test, made weight gains as good as those of the uninoculated controls and had very few lesions of *E. acervulina* at necropsy. A 4-day treatment beginning 3 days after a single inoculation of oocysts prevented symptoms and greatly reduced oocyst output but did not prevent some retardation in weight gains.

5. Chickens treated with sulfaguanidine for 4 to 7 days, beginning on the 4th or 5th day after a single inoculation, exhibited the same symptoms as the inoculated controls but they showed some benefit, as indicated by more rapid recovery.

6. Treatment with 0.5 per cent sulfaguanidine for 4 or 5 days, beginning after clinical symptoms appeared, did not eliminate the parasites from the host; nor did a 4-day treatment, beginning at the time of inoculation, destroy the parasites.

7. Birds reinoculated 18 days after cessation of treatment showed a marked resistance to reinfection.

LITERATURE CITED

DICKINSON, E. M. 1939 The effects of variable dosages of sporulated *Eimeria acervulina* oocysts on chickens. Poultry Sci. **18**: 404.

DICKINSON, E. M. 1941 Idem. Poultry Sci. **20**: 413–424.

DICKINSON, E. M. AND SCOFIELD, R. H. 1939 The effect of sulphur against artificial infection with *Eimeria acervulina* and *Eimeria tenella*. Poultry Sci. **18**: 419–431.

FARR, M. M. AND ALLEN, R. 1942 Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens. J. Amer. Vet. Med. Ass. **100**: 47–51.

JOHNSON, W. T. 1931 Effect of five species of *Eimeria* upon egg production of Single Comb White Leghorns. J. Parasit. **18**: 122.

LEVINE, P. P. 1941 The coccidiostatic effect of sulfaguanidine (sulfanilyl guanidine). Cornell Vet. **31**: 107–112.

LEVINE, P. P. 1943 The selective action of sulfaguanidine on avian coccidia. J. Parasit. **29**: 362–363.

TYZZER, E. E. 1929 Coccidiosis in gallinaceous birds. Am. J. Hyg. **10**: 269–383.

TYZZER, E. E., THEILER, H. AND JONES, E. E. 1932 Coccidiosis in gallinaceous birds. II. A comparative study of species of *Eimeria* of the chicken. Am. J. Hyg. **15**: 319–393.

RESEARCH NOTES

FURTHER NOTE ON LEECH INFESTATION IN MAN

In 1941 the writer reported the finding of leeches in the human nose from Lotien, Kweichow Province and Chaotung, Yünnan Province, China, (Chinese Med. J., 60: 241–243). During the winter of 1944, the Chaotung specimen was lost. The three Lotien specimens were brought to the United States in 1947 and at the suggestion of Prof. Ernest Carroll Faust were sent for identification to Prof. J. Percy Moore, University of Pennsylvania. These were determined as *Dinobdella ferox* (Blanchard), all of them being immature, and therefore not fully characterized.

In his letter to Dr. Faust (dated May 27, 1947), Dr. Moore made a statement regarding his unpublished observations on this species in the Orient, as follows:

"*Dinobdella ferox* (Blanchard) is widely distributed in India, Burma, Southeast China and Formosa and is a serious pest of domestic and some wild mammals. It is known to attack man by taking up its abode in the air passages. You will find about all that was known of it at the time in the volume on Hirudinea in the Fauna of British India, 1927 (Moore, Arhynchobdellae, pp. 175–185). During my visit to India in 1930–31, I discovered that it breeds at springs, wells and small streams (and probably some larger bodies of water) frequented by cattle, horses and other mammals. The egg capsules are deposited in the mud and the newly hatched young, $\frac{1}{2}$–$\frac{3}{4}$ in. long, abound in the water on aquatic plants, etc. If one places a hand in the water many of the little leeches instantly attach to it and rapidly loop up the arm. The same happens to the muzzle of a cow or horse or the face of a man drinking leech-infested water, and the young leeches quickly enter the nostrils or mouth and attach themselves to the wall of the respiratory passages, usually far back in the pharynx or larynx. They grow rapidly and reach a large size and often do much damage."

Although Dr. Moore did not state the exact localities in Southeast China where this leech has been found, yet it seems to be widely distributed south of the Yangtze River. Since it is important both in veterinary and medical sciences, a thorough study of its distribution, biology and life history in China seems desirable.

The writer is indebted to Prof. Moore for identifying the specimens and the information regarding this species, which he has generously granted permission to quote.—Ta-Hsiung Chin, *National Kweiyang Medical College, Kweichow, China.*

ON THE PARASITISM OF THE LEECH, *PISCICOLA SALMOSITICA* MEYER, 1946

In a recent publication by the writer (1946. J. Parasit. **32**(5):467–476) the steelhead trout (*Salmo gairdneri gairdneri*) was given as the host for *Piscicola salmositica*. Mr. Leo Shapovalov, California State Division of Fish and Game, has kindly called attention to the fact that the proper spelling of the scientific name of the steelhead is *Salmo gairdnerii gairdnerii* rather than *S. gairdneri gairdneri*. He also pointed out that in the case of the Soos Creek specimens, an additional host species was involved, since all of the salmon of the Pacific Coast belong to the genus *Oncorhynchus.*

Dr. James E. Lynch, School of Fisheries, University of Washington, in a written communication dated 14 March 1947, informed me, the salmonids found in the vicinity of the Soos Creek Hatchery of King County, Washington, in addition to the steelhead, include the following species: the silver salmon (*Oncorhynchus kisutch*), the king salmon (*O. tshawytscha*), and the chum salmon (*O. keta*). In the same communication Dr. Lynch stated, "The *Piscicola salmostica* from the vicinity of the Soos Creek Hatchery, . . ., were either collected from the gravel in the bottom of the creek, or taken from the surface of salmon, but mostly were collected from the bottom. . . . Since the fish will die after spawning, anyway, the hatchery men kill the fish prior to salvaging the eggs. The numerous live fishes collected in the trap, plus the blood, spilled eggs, etc., in the water, apparently attract the leeches from a long distance downstream."

Since *Piscicola salmositica* is not a permanent parasite and agrees with most piscicolids in not being host-specific, the omission of any particular host fish is relatively unimportant. However the writer welcomes the opportunity to include the additional salmonid hosts as well as to correct the spelling of the scientific name of the steelhead.—Marvin C Meyer, *Department of Zoology, University of Maine, Orono, Maine.*

FURTHER STUDIES ON RADIOIRON IN AVIAN MALARIA

In a previous communication (Thompson, McGinty, Bush and Wilson, J. Inf. Dis. 83: 23, 1948) canaries and ducks were treated with radioiron (Fe 55, 59) prior to infection with malarial

parasites. Although conditions were favorable for utilization of 10–30% of the injected radioiron in hemoglobin formation, the resulting radiation had no influence on the morphology of the plasmodia or on the course of the initial infections. This was attributed to the low specific activity of available samples of radioiron which permitted the attainment of only 0.1 microcurie of radioactivity per ml. of blood.

Recently radioiron (Fe 55, 59) of approximately 10-fold greater specific activity than that used in the previous work has become available. The present communication describes the results of an attempt to alter the initial course of blood-induced *Plasmodium cathemerium* infections in canaries with this material. The procedures were identical with those used earlier except that birds were not kept on an iron-deficient diet. Details of this study are summarized in the accompanying table. By references to these data it is evident that levels of radioactivity as high as 1.1 microcurie per ml. of blood were without effect on the initial infections. Furthermore, these levels of radiation were without effect on the morphology and staining reactions of the parasites.— PAUL E. THOMPSON, D. A. McGINTY, MARY L. WILSON AND ANITA BAYLES, *The Research Laboratories, Parke, Davis and Company, Detroit, Michigan.*

	Bird no.	Days treated before infection	Total amount Fe injected mg./kg.	% Fe uptake in blood cells	Radioactivity* μc/ml. blood	Parasites/10,000 RBC on days after infected					
						2	3	4	5	6	7
Radioiron Treated	136	14	137.2	10.5	1.0	< 30	< 30	< 30	400	2250	4000
	140	11	118.4	..†	..†	340	2600	4100	2400	2730	3300
	147	11	118.4	11.4	1.1	280	1600	2050	Died		
	149	11	126.2	8.2	0.7	352	1500	2550	Died		
	150	10	98.5	14.3	1.0	360	2550	1900	1830	1130	1600
	151	9	81.2	9.3	0.6	410	1700	1270	1500	870	700
	153	9	100.6	8.8	0.6	585	1300	3200	2950	2750	Died
	155	9	76.7	12.9	0.9	1112	1750	1550	1150	1150	1400
	157	9	92.0	9.2	0.6	200	1200	2150	2550	1750	1300
						205‡	749‡	936‡	1563‡	1651‡	1731‡
Natural Iron Treated Controls	135	14	143.0			630	3350	2450	540	1650	1650
	141	8	93.8			360	2300	4100	2400	Died	
	144	14	133.4			< 30	< 30	< 30	200	1520	3750
	148	14	143.0			165	1500	2450	1700	1366	1100
						78‡	328‡	396‡	815‡	1508‡	1895‡
Untreated Controls	162						1200	2050	1330	1140	1800
	163						1370	2800	2100	2000	930
	164						1150	2600	2300	1960	1200
	165						1400	2800	2630	1360	1050
	166						1120	1850	1850	1500	1080
							1243‡	2386‡	1991‡	1556‡	1179‡

* Determined one day before inoculation of parasites.
† Sample lost.
‡ Geometric mean.

PENETRATION CHARACTERISTICS OF *SCHISTOSOMA MANSONI* CERCARIAE

There is little exact information in the literature concerning the length of time required for penetration of schistosome cercariae. Times from 18 seconds to 1 hour have been reported by various investigators. The need for accurate information on the penetration characteristics of *Schistosoma* cercariae brought about this study.

In this work freshly passed cercariae, apparently in excellent condition, were under continuous microscopical observation (40×) from the time they were placed on the skin until they disappeared below the surface or for a period of 15 minutes in the case of those not penetrating. In the first part of the study observations were made on cercariae of *Schistosoma mansoni*, Venezuelan strain from laboratory raised Puerto Rican *Australorbis glabratus,* which were placed on the skin of hamsters. The six hamsters, weight 95–140 grams, were used in rotation for the experiment during periods of four hours on three successive days. The hamsters were relaxed with Nembutal and a drop of water containing freshly shed cercariae was placed on the clipped skin of the lower abdomen. The exposed area was illuminated from one side by a strong beam of light. The average length of time of penetration, that is, the time from placing the cercaria on the skin until the body disappeared below the surface, for the 75 penetrating cercariae observed, was 4.3 minutes with a range of from 2–15 minutes. Six additional cercariae were observed but did not penetrate in the 15 minutes period.

In the second part of the study cercaria of *S. mansoni* were observed as they penetrated six white laboratory mice weighing 20–30 grams. The mice were restrained in a supine position by

taping to a piece of plate glass. The head was turned to the side by a piece of tape placed diagonally across the cheek. While the ear was pulled firmly away from the head by means of forceps, a piece of 1 inch by 2 inch tape, containing a hole just a little smaller than the ear, was placed over the ear so that a large portion of the external ear was visible. Cercariae of *S. mansoni* from the same source as those in the previous experiment were observed from the time the drop of water containing them was placed on the mouse's ear until they had penetrated and disappeared. Illumination was provided as in the previous part of the study. The length of time for penetration of the mouse's ear ranged from 2.5–8 minutes with an average of 3.6 minutes for the 50 penetrating cercariae observed. An additional 5 cercariae were observed but did not penetrate in 15 minutes. The 6 mice were used in rotation for the observations during 4 hour periods on 3 successive days.

When placed on the skin of both animals, the cercariae usually swam around for about 1 minute, then crawled along on the skin in a measuring worm manner pulling themselves by means of the oral sucker. At the beginning of the penetration the cercariae were perpendicular to the skin, being attached by the oral sucker. The tail waved violently but usually did not break from the body until after the body had completely penetrated. Once penetration had started, it proceeded rather rapidly. The cercariae on the mouse's ear had a tendency to crawl under the overhang of the tape or out to poorly illuminated areas. Possibly this suggests negative phototaxis.

These tests were performed in the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland. The author wishes to express her appreciation for the use of the facilities and materials and for personal assistance from members of the Section of Helminthic Diseases.—Dorothy J. Hitchcock, *Department of Bacteriology and Public Health, Michigan State College.*

THE OCCURRENCE OF NEOTROPICAL MITES IN THE UNITED STATES

A recent article by Turk (1947, Ann. & Mag. Nat. Hist. Ser. II, 13, 347) on parasitic mites has pointed up two things that have been known here for some time but have never been published and we feel that the information should be made generally available.

1) In commenting on the genus *Gigantolaelaps* Fonseca, Turk mentioned that it contains very large neotropical mites, confined as far as is known to Brazil and the Argentine. We have records of the genus from the rice-rat, *Oryzomys palustris* from Texas and south Georgia, but the mite probably occurs throughout the range of the rice-rat. In south Georgia 46 specimens of *Gigantolaelaps* were removed from 14 of the 28 swamp rice-rats collected. Rice-rats were taken during the months of January, February, March, April, October, November, and December and one or more was found to be infested with *Gigantolaelaps* during each of these months except March when only one rat was examined. The species is apparently new and a paper describing it is in preparation. It appears to be fairly common and is probably an indigenous species.

Another mite that is considered neotropical but which is quite common in the United States is *Neoichoronyssus wernecki* (Fonseca) (= *Liponyssus wernecki* Fonseca) (1941, Ciencia, 2, 264). This mite was described from *Didelphis* spp. in Brazil. It is common on species of *Didelphis* from southern United States. Mr. G. C. Menzies of the Texas State Health Department has records from various parts of Texas, Mr. O. E. Hunt has taken it many times on opossums from the vicinity of Houston, and the U. S. Public Health Service has many records from south Georgia where 189 specimens were removed from 29 (10.9%) of the 266 *Didelphis floridana* collected. The largest percent infestation of opossums with one or more of these mites (27.59%) occurred in May. Infestation was found in all months except July, August, September, and November. It frequently is associated with *Bdellonyssus bacoti* (Hirst) (= *Liponyssus bacoti* (Hirst).

Liponyssus hematophagus Fonseca (1935, Mem. Inst. Butantan, 10, 43), described from bats in Brazil, has been taken in Georgia on *Tadarida cynocephala* and *Myotis lucifugus*. This of course is not surprising in view of the fact that bats are migratory.

2) In describing *Macrolaelaps bhutanensis* Turk, (ibid) wisely stated that the inclusion of the species in that genus is purely provisional. From the description and figure given, it would seem that his species is a *Laelaps sensu strictu* and closely related to *Laelaps nuttalli* Hirst. Turk's error lay in accepting the characters given in Ewing's key (1929, Manual of External Parasites, Baltimore), which separates the laelaptine mites on the basis of the presence or absence of a brush of small setae at the base of the digitus mobilis. Actually, all species of *Laelaps sensu latus* have this character and it is therefore worthless as a differentiating feature.—Harvey B. Morlan, *S. A. Sanitarian (R), Communicable Disease Center, Public Health Service, Thomasville, Georgia;* and R. W. Strandtmann, *formerly with the University of Texas, now with the Department of Biology, Texas Technological College, Lubbock.*

CAPILLARIA ANNULATA (MOLIN, 1858) FROM THE MUCOSA OF THE MOUTH OF THE DOMESTIC FOWL, GALLUS DOMESTICUS (LINN.)

Adult specimens of a nematode identified as *Capillaria annulata* (Molin, 1858) were found imbedded in the mucosa of the floor of the mouth of three adult chickens autopsied by the author in 1947.

The birds were grade Cornish from a backyard flock at Auburn, Alabama. All had heavy infestations of *C. annulata* throughout the crop and length of the oesophagus; the affected area extended from the lower oesophagus just above the proventriculus anteriorly to in front of the base of the tongue. A total of 17, 30, and 32 worms were counted in the mouths and pharynges of three birds which harbored 156, 125, and 199 *C. annulata* in their crops and oesophagae, respectively. The lengths of the worms (2–3 cm) in the mouth were about the same as those present in the upper oesophagus; however, in both these locations they were usually not over one-half to two-thirds the length of those present in the crop. There was evidence of caseation, erosion, and inflammation of the infested buccal mucosa in the two birds with the heaviest mouth infestations. Although the sides and roof of the mouth, pharynx, and larynx were carefully examined, no worms were found in those areas.

Two other birds from the same source were examined; it is of interest that these did not have such heavy infestations in their crops and oesophagae and had no worms in the mouth. It may be that the presence of *C. annulata* in the mucosa of the mouth is associated with the degree of infection in the normal habitat. As far as the author is aware, this is the first report of *C. annulata* living in the mouth. However, its presence in the mouth may not be rare, since, at post-mortem, that area is not usually examined carefully. That *C. annulata* infections may so affect birds that they refuse grain and accept only soft feed has been noted by earlier investigators. The present observations on buccal infestations may be of significance in that connection. The specific identification and presence of this worm in the buccal cavity of the chicken were confirmed by Dr. E. E. Wehr, *Zoological Division, B. A. I., Beltsville, Maryland.—*S. A. Edgar, *Department of Poultry Husbandry, Alabama Agricultural Experiment Station, Auburn, Alabama.*

ADDITIONAL RECORD OF THE OCCURRENCE OF THE ACANTHOCEPHALAN, EOCOLLIS ARCANUS VAN CLEAVE

The report herein of the occurrence of *Eocollis arcanus* marks the second record of this unusual member of the family Neoechinorhynchidae. Van Cleave (1947. The Eoacanthocephala of North America, including the description of *Eocollis arcanus,* new genus and new species, superficially resembling the genus *Pomphorhynchus.* J. Parasit. 33: 285–296) first described the species from *Lepomis macrochirus* Raf. (the Bluegill) from the Ohio River at Shawneetown, Illinois, and *Pomoxis nigro-maculatus* (Le Sueur) (the Black Crappie) from Horseshoe Lake near Cairo, Illinois. The present record dates from March 23, 1936, when a total of five specimens were taken from *Pomoxis annularis* Raf. (the White Crappie) and *Pomoxis nigro-maculatus* (Le Sueur) both of which were collected from the Illinois River at Havana, Illinois.

There are three males and two females in the present collection, all of which are obviously not yet sexually mature. The single egg mass in the females has not yet broken while in the males the cement glands are apparently not yet fully developed. The forebody is elongated into a false neck as originally described by Van Cleave, although the prominent bulb mentioned in the original description is wholly lacking in all specimens. The males have 5 dorsal and 1 ventral subcuticular nuclei, while the females have 6 dorsal and 1 ventral. In all other respects the specimens at hand are in agreement with the description as given by Van Cleave.—David R. Lincicome, *Department of Microbiology, University of Wisconsin Medical School, Madison.*

THE CULTIVATION OF THE SMALL RACE OF ENDAMOEBA HISTOLYTICA WITH A SINGLE SPECIES OF BACTERIA

The occurrence of two distinct races of *Endamoeba histolytica,* based on size, was substantiated by Sapero, Hakansson, and Louttit (1942, Am. J. Trop. Med., 22, 191–208) who were of the opinion that physiological differences also exist between the large and the small race and are reflected by differences in culturability and pathogenicity. In this laboratory, the question has arisen as to whether there might also be differences in the antigenicity of the large and the small race. For comparative tests, cultures of both races are necessary for the preparation of antigen. Efforts have been made to establish the small race in the same type of culture medium used for the large race.

By means of the micromanipulator, it was possible to obtain growth *in vitro* of the small race of *E. histolytica* in association with a single species of bacterium, organism *t*. The cultures were initiated from cysts obtained from the stool of a nine-year old white girl with no overt symptoms of amoebiasis. To establish the cultures, several hundred cysts were picked from the fecal material with the micropipette, washed free from bacteria through repeated changes of sterile Locke's solution and seeded, ten at a time, into tubes of Locke-egg medium containing rice flour and a fresh inoculum of organism *t*. On incubation at 37° C., 7 of 10 tubes became positive for amoebae, 4 in 6 days, and 1 each in 7, 8, and 9 days. One of the early positive cultures was selected for continuation of the strain and has been in cultivation since March 22, 1948.

Evidence that the cultures so established are pure for *E. histolytica* and are of the small race only is based on the following points. Repeated examination of the fecal material from the patient demonstrated only one type of organism represented by small cysts which showed typical nuclei when stained with iodine and characteristic chromatoid bodies when observed in water. The average diameter of 100 cysts stained with iodine was 7.3 microns, the mode 7.2, and the extremes 5.4 and 9.9 microns. In addition, by the microisolation technique, it was possible to observe that all cysts used for inoculation of medium were of the small variety. The trophozoites in the cultures exhibited characteristic nuclei when stained with hematoxylin by the cover-slip technique of Dobell (1942, Parasitology, 34, 101–112). The average diameter of 100 rounded trophozoites stained with hematoxylin at 48 hours of incubation was 7.6 microns, the mode 6.8, and the extremes 3.0 and 11.9; with the iodine stain, also at 48 hours incubation, the average diameter was 7.6, the mode 7.2, and the extremes 5.4 and 10.8 microns.

With organism *t*, the small race can be maintained at 72- and 96-hour intervals of transfer. The trophozoites appear as active as the trophozoites of the large race; they ingest starch and, like the large race, do not encyst. The failure of encystation in cultures of amoeba with single species of bacteria has also been observed by Dobell (1947, Liber Jubilaris J. Rodhain, 201–211).

A phenomenon noted by us in cultures of the large race has also been observed in the small race and is concerned with the effect of the amoebae on the rice flour of the medium. The flour is prepared from unpolished rice and when examined microscopically appears as coarse particles which are composed of intracellular starch grains enveloped by gluten. Very few discrete starch grains are visible in the material. This appearance of the flour is also characteristic in cultures in which only organism *t* is growing. On the other hand, in cultures in which amoebae are growing in addition to the bacterium, a profusion of discrete starch granules is seen. This phenomenon has also been observed by Anderson and Hansen (1947, Liber Jubilaris J. Rodhain, 47–63) and is evidence of enzymatic activity on the part of the amoeba to liberate the starch grains from the protein envelope. This evidence is strengthened by the demonstration that ficin, a proteolytic enzyme prepared from a species of fig, has the same effect on the rice flour.

With the cultivation of the small race of *E. histolytica* in a known biotic environment, means are provided for studying problems in antigenicity of this race. A known biotic environment is also a means of establishing racial stability, a question which has recently been reopened by Meleney and Zuckerman (1948, Am. J. Hyg., 47, 187–188) who reported reversal of form of the small race in culture. With organism *t*, as well as with the addition of other known bacteria, cultures can be studied for the possible effect of the flora on the size and other characteristics of the amoeba.—Lucy V. Reardon and Ida Louise Bartgis, *Laboratory of Tropical Diseases, National Institutes of Health, Microbiological Institute, U. S. Public Health Service, Bethesda, Maryland.*

ANTAGONISM OF CALCIUM PANTOTHENATE FOR THE ANTIMALARIAL EFFECT OF PANTOYLTAURYLAMIDE

Brackett and co-workers (1946, J. Parasit. 32: 5) reported the antagonism between calcium pantothenate and certain analogues of pantothenic acid with respect to their antimalarial activity against *Plasmodium gallinaceum*. Since our own work on the antagonism of such substances appeared in the comparatively inaccessible reports of the Office of Scientific Research and Development (Malaria Report #28, 1943), it seemed worthwhile to publish the present note.

The antagonism between calcium pantothenate and pantoyltaurylamide (referred to hereafter as PTA) may be seen in table 1. In this table are found parasite counts of groups of ten chickens on the sixth day after infection which were treated intravenously with daily doses of 400 mg. per kilo PTA alone and together with 100, 50 and 25 mg. per kilo calcium pantothenate, respectively. As controls, a fifth group was treated with 10 mg. per kilo quinine dihydrochloride, a sixth one with 10 mg. per kilo quinine dihydrochloride and 100 mg. per kilo calcium pantothenate, and a seventh one with no drug. Treatment in all groups was initiated on the day after infection and consisted of 6 doses.

The antimalarial effect of 400 mg. per kilo PTA was completely nullified by 100, 50 or 25

mg. per kilo calcium pantothenate. In fact, these three groups had more parasites than the untreated controls. The effect of 10 mg. per kilo quinine was unaffected by 100 mg. per kilo calcium pantothenate.

The history of this group of drugs is interesting because it was discovered deductively (see Blanchard and Schmidt in "A Survey of Antimalarial Drugs 1941–1945", page 138). The idea of using analogues of pantothenic acid was based on Trager's finding (J. Exp. Med. 1943, 77: 411) that pantothenate prolonged the survival of *Plasmodium lophurae* in vitro. Trager suggested in 1942 to the Malaria Conference that inhibitors of pantothenic acid be tested. Acting on this suggestion, Dr. W. M. Clark and Dr. W. H. Taliaferro induced Dr. J. B. Koepfli to underake the synthesis of such substances. The compounds prepared by Dr. Koepfli and his co-workers were tested at the University of Chicago in 1943 (Malaria Report #14) and pantoyltaurylamide was found to be active (see Survey of Antimalarial Drugs). Because of the relatively large amounts of PTA required to suppress the infection (about 40 times as much as of quinine) and the failure to find a more active analogue, interest in this type of compound lagged until Wooley (1944, Science 100: 579) reported that phenylpantothenone is a competitive antagonist of pantothenic acid. Wooley supplied the Survey with enough of this substance to

TABLE 1.—*Antagonism of Calcium Pantothenate and Pantoyltaurylamide in* Plasmodium gallinaceum *Infection*

Drugs* administered mg/kilo/day			Average per cent of parasitized red cells on the sixth day of infection in 10 chickens in each of 7 groups										Mean % red cells parasitized with standard error
PTA	CP	Q											
400	..	..	<1	<1	<1	<1	<1	<1	11	3	2	1	2.5 ±1.1
400	100	..	80	39	75	86	60	64	57	40	51	57	60.9 ±5.0
400	50	..	88	76	73	68	87	72	87	85	55	60	75.1 ±3.4
400	25	..	87	76	61	62	65	65	89	62	85	48	70.0 ±4.3
..	..	10	<1	<1	0	<1	0						0.1
..	100	10	0	0	0	<1	<1						0.01
Control			14	22	77	43	56	33	8	50	41	71	41.5 ±7.2

PTA, pantoyltaurylamide; CP, calcium pantothenate; Q, quinine.
* Injected intravenously once a day for six days.
Blood induced infections started by injecting intravenously one million parasites into each two-week old chick.

conduct tests. It was found to be about 0.8 times as active as quinine against *P. gallinaceum* in tests carried out at the University of Michigan and twice as active as quinine against *P. lophurae* at the University of Chicago (A Survey of Antimalarial Drugs, J. W. Edwards, Ann Arbor, 1946).

More active compounds were found in SN14620, SN14621 and SN14622 which were synthesized by Winterbottom and co-workers and tested by Brackett and co-workers (1946, J. Parasit. 32: 5).—WILLIAM CANTRELL, *Departments of Bacteriology and Parasitology and Department of Pharmacology, The University of Chicago.*

This work was done under a contract between the Office of Scientific Research and Development and the University of Chicago.

ON THE STATUS OF *PLEORCHIS MOLLIS* (LEIDY, 1856) STILES, 1896 (TREMATODA)

The trematode genus *Pleorchis* was named by Railliet in 1896 for *Polyorchis* Stossich, 1892 (preoccupied). *Pleorchis polyorchis,* the type species, is from the intestine of a marine fish, *Corvina nigra,* at Trieste. Other species are *Pleorchis sciaenae* Yamaguti, 1938 from *Sciaena albiflora* in the East China Sea, and *Pleorchis americanus* Lühe, 1906 from *Cynoscion regalis* at Woods Hole, Massachusetts. This latter species has had a rather involved history. Linton (1901) first reported it as *"Distomum polyorchis* Stoss." (Bull. U. S. Fish Comm. for 1899, p. 460). Lühe (1906, Rep. Gov. Ceylon Pearl Oyster Fish, Gulf of Manaar, pt. 5, p. 103) recognized its differences from the European species and named it *Pleorchis americanus.* Yamaguti (1938, Studies on the helminth fauna of Japan, Part 21, Kyoto, Japan, p. 57), evidently unaware of Lühe's name, gave it the name *Pleorchis lintoni.* In 1894, Stiles and Hassall transferred *Monostomum molle* Leidy, 1856 to the subgenus *Polyorchis* (=*Pleorchis*). Their 1895 (Vet. Mag., 1 (11) : 737–741) description of the supposed *M. molle* clearly indicates they were dealing with a species of *Pleorchis* and the name later became *Pleorchis mollis* (Leidy, 1856) Stiles, 1896. A study of Stiles and Hassall's description and figures shows that this species agrees with *Pleorchis americanus,* assuming that the double nature of the testes was overlooked as would be

the case without study of cross-sections. Suspicion that something is wrong arises when it is noted that Leidy collected his *Monostomum molle* from the lungs of a turtle, that he called it a monostome, and that his two poorly preserved specimens were 9 lines (=19 mm.) in length. Although this description is very incomplete it suggests, today, the common lung monostome of turtles (*Heronimus*). Certainly, it does not agree at all with *Pleorchis* which is very clearly a distome only 3 to 7 mm. in length and parasitic in the intestine of marine fishes. It seems to me an obvious error of some kind was made in identifying the *Monostomum molle* of Leidy as *Pleorchis*. A possible clue lies in the fact that in Leidy's same list of parasites (Leidy, 1856, Proc. Acad. Nat. Sci., 8 : 42–58) is a distome, *Distomum incivile*, reported from a marine fish, *Leiostomus obliquus*—now *L. xanthurus*. The dimensions of *D. incivile* agree with those of *Pleorchis americanus* and with the trematodes described by Stiles and Hassall, and the host is related to the genus *Sciaena* from which one species of *Pleorchis* is definitely known. In Leidy's list "*Monostomum molle*" is number 18 and "*Distomum incivile*" is number 28.

In view of the above facts, the following names are considered as synonyms of *Pleorchis americanus:* the "*Distomum polyorchis* Stoss." of Linton, 1901; *Pleorchis lintoni* Yamaguti, 1938 (new synonymy) ; the "*Monostomum molle* Leidy" of Stiles and Hassall (1894 and 1895) which they named "*Distoma (Polyorchis) molle* (Leidy, 1856)" and which Stiles in 1896 transferred to *Pleorchis*. The actual *M. molle* is probably *Heronimus chelydrae*, although practically no description was given and unless more authentic type specimens are located the name should be considered a *nomen nudum*. It is suggested that confusion has resulted possibly by a mislabeling of Leidy's material or by misinterpretation of hand-written figures.—HAROLD W. MANTER, *University of Nebraska, Lincoln, Nebraska.*

THE TREMATODE *CATHAEMASIA PULCHROSOMA* (TRAVASSOS, 1916) N. COMB. FROM THE BODY CAVITY OF A KINGFISHER (*MEGACERYLE ALCYON*) IN NEBRASKA

Travassos (1916, Brazil-Medico 30 (40) : 313–314) described the genus and species *Pulchrosoma pulchrosoma* from the abdominal cavity (air sacs) of *Ceryle torquata* in Brazil. The species was later redescribed and illustrated (Travassos, 1928, Mem. Inst. Oswaldo Cruz 21 : 343–372). The descriptions of this trematode seem to have been overlooked by subsequent authors dealing with related species. One explanation might be that *Pulchrosoma* was erroneously listed as a nematode in the Zoological Record for 1916 (Vol. 53, 1919) and in Neave's Nomenclator Zoologicus. The Zoological Record for 1926 lists it correctly as a trematode. No differences of generic value seem to distinguish *Pulchrosoma* from *Cathaemasia* Looss, 1899 and it is here considered a synonym.

Several years ago a single specimen of a *Cathaemasia* species was collected from the body cavity of a belted kingfisher, *Megaceryle alcyon,* taken near Lincoln, Nebraska. It is considered to be the same as the Brazilian species (*C. pulchrosoma*) from *Megaceryle torquata*. The only differences are that my specimen has a slightly smaller body (12.1 mm. as compared with 15 to 20 mm.) and somewhat smaller eggs (116 to 131 by 66 to 72 μ as compared with 140 by 74 μ). Harwood (1936, Jour. Tenn. Acad. Sci. 11 (4) : 251–256) has redescribed *Cathaemasia reticulata* (Wright, 1879) Harwood, 1936 which he collected from the body cavity of *Megaceryle alcyon* in Tennessee. Although Harwood felt that the trematode probably came from the intestine, it should be noted that specimens collected by Wright, Travassos, Harwood, and myself were all in the body cavity and probably inhabited the air sacs.

C. pulchrosoma is very similar to *C. reticulata* differing chiefly in that the posttesticular space is much longer and the vitelline follicles there meet medially ; in addition, the ovary is slightly lobed, the body and eggs somewhat larger, and the acetabulum is slightly larger compared with the oral sucker. On my specimen, as in the case of *C. reticulata,* some scales occur anteriorly on both the dorsal and ventral surfaces.—HAROLD W. MANTER, *University of Nebraska, Lincoln, Nebraska.*

"MEDICHROME" SERIES MT

TRADE MARK REGD. U. S. PAT. OFF.

TROPICAL DISEASES

A series of approximately 160 2 x 2″ (35 mm.) Kodachrome transparencies (lantern slides) made with the cooperation of Dr. Henry E. Meleney, Dr. Harry Most and Dr. Dominic DeGiusti, Department of Preventive Medicine, New York University College of Medicine.   Slides Nos. MT 45, 46 and 47 are from the Army Medical Museum, Washington, D. C.   The series consists of photomicrographs of parasites and insect carriers of the following groups:

VIRUS DISEASES

BACTERIAL DISEASES

RICKETTSIAL DISEASES

SPIROCHETAL DISEASES

FUNGUS DISEASES

PROTOZOAN DISEASES

Malaria

Trypanosomiasis

Leishmaniasis

Intestinal Flagellates

Intestinal Ciliates

Intestinal Amebae

E. Histolytica

Others

HELMINTH DISEASES

Trematodes

Fascioliasis

Clonorchiasis

Paragonimiasis

Schistosomiasis

Cestodes

Nematodes
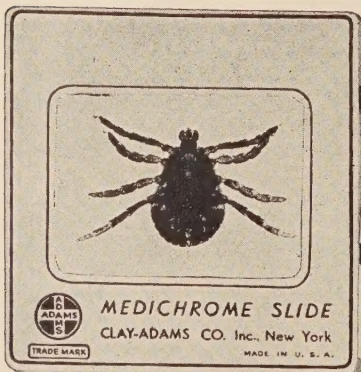
Trichinosis

Hookworms

Filaria

ARTHROPODS

Ticks and Mites

Bugs

Blood-sucking Flies

Mosquitoes

Sandflies

Black Flies

Muscid Flies

Lice

Fleas

MT 235.   Echinococcus granulosis toto mount 30X

KODACHROME

MEDICHROME SLIDE

CLAY-ADAMS CO. Inc., New York

MADE IN U. S. A.

MT 284.   Dermacentor, sp.

$1.00 per slide, bound in ADAMS Slide Binders

$.80 per slide in cardboard readymounts

Orders for 50 slides, less 5%;

for 100 slides or more, less 10%
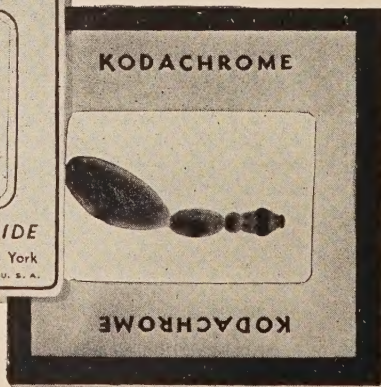
CLAY-ADAMS COMPANY, INC.

141 EAST 25th STREET · NEW YORK 10

Showrooms also at 308 West Washington Street, CHICAGO 8, ILL.